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Hot Topics in Infection and Immunity in Children IX

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Preface

The ninth 'Infection and Immunity in Children' (IIC) course was held in July 2011 at St Catherine's College, Oxford, UK. This book, the ninth in the series, comprises chapters based on presentations made at that course. The series provides succinct and readable updates on virtually every topic of relevance to Paediatric Infectious Diseases (PID).

The tenth edition is currently in preparation following the 2012 course, which once again had a programme delivered by renowned top-class speakers. The programme for the eleventh course, planned for 26–28th June 2013, is being finalised as this book goes to press.

PID has emerged as a powerful discipline for the improvement of child health in Europe and worldwide over the last quarter of a century. The European Society for Paediatric Infectious Diseases (ESPID) now holds the largest annual conference in PID in the world and sponsors a wide range of educational activities for trainees and specialists. Among them is the Oxford IIC course, which, with other ESPID-sponsored activities, is an integral part of the two-year Oxford Diploma in PID. This course began in 2008 and continues to enrol trainees from all over Europe, being the first recognised academic qualification of any kind in PID outside North America.

The future of PID promises to be eventful, challenging and fascinating, as new infections emerge, old infections evolve and new diagnostic techniques, treatments and vaccines become available. There is much that is new to learn about and we hope this book will provide a further useful contribution to the materials available to trainees and practitioners in our important and rapidly developing field.

Melbourne, Australia	Nigel Curtis
Bristol, UK	Adam Finn
Oxford, UK	Andrew J. Pollard

Acknowledgments

We thank all the speakers from the 2011 'Infection and Immunity in Children' (IIC) course who gave their time both to attending the meeting and to contributing presentations, discussion and chapters for this book. We also thank their co-authors, many of whom are trainees who have also attended the course on one or more occasions.

Since its early days, the course has been organised by Sue Sheaf. Its continuing and growing success is in large part due to her efforts, which include all the necessary liaison with the faculty, the handling of applications and bursaries for all the delegates, working with the college with respect to all practical arrangements for the course, the audiovisual team, the social programme and, of course, cajoling us, the organisers, into doing our part, to name but a few. Without Sue, the Oxford IIC course would not be what it is and we cannot thank her enough.

Another difficult task is the administration of the production of successive editions of this book. As anyone who has ever been involved in putting together a publication of this kind will know, this is a major undertaking requiring a skilled and dedicated team of professionals. Pamela Morison is that team. Careful copyediting and correction of the materials provided by the authors, editors and publishers is essential to ensuring a high-quality final product and Pam is the person who does this work. She also has to persuade all these parties to deliver the materials in a timely fashion, which Pam does with the right mix of persuasiveness and insistence. For Pam's good-natured approach, diplomatic skills and all her hard work we thank her and gratefully share with her the credit for this book's production.

We thank the European Society for Paediatric Infectious Diseases (ESPID) for consistent support and financial assistance for this and previous courses and also for providing bursaries, which have paid the costs of many young ESPID members' attendance. We also acknowledge the recognition given to the course by the UK Royal College of Paediatrics and Child Health (RCPCH).

Finally, we are grateful to the staff of St Catherine's College, Oxford, UK where the course was held and to several pharmaceutical industry sponsors who generously offered unrestricted educational grants towards the budget for the meeting.

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Innovation for the 'Bottom 100 Million': Eliminating Neglected Tropical Diseases in the Americas

Peter J. Hotez, Eric Dumonteil, Michael J. Heffernan and Maria E. Bottazzi

Abstract

An estimated 100 million people in the Latin American and Caribbean (LAC) region live on less than US$2 per day, while another 46 million people in the US live below that nation's poverty line. Almost all of the 'bottom 100 million' people suffer from at least one neglected tropical disease (NTD), including one-half of the poorest people in the region infected with hookworms, 10 % with Chagas disease, and up to 1–2 % with dengue, schistosomiasis, and/or leishmaniasis. In the US, NTDs such as Chagas disease, cysticercosis, toxocariasis, and trichomoniasis are also common among poor populations. These NTDs trap the poorest people in the region in poverty, because of their impact on maternal and child health, and occupational productivity. Through mass drug administration (MDA), several NTDs are on the verge of elimination in the Americas, including lymphatic filariasis, onchocerciasis, trachoma, and possibly leprosy. In addition, schistosomiasis may soon be eliminated in the Caribbean. However, for other NTDs including hookworm infection, Chagas disease, dengue, schistosomiasis, and leishmaniasis, a new generation of 'anti-poverty vaccines' will be required. Several vaccines for dengue are under development by multinational pharmaceutical companies, whereas others are being pursued through non-profit product development partnerships (PDPs), in collaboration with developing country manufacturers in Brazil and Mexico. The Sabin Vaccine Institute PDP is developing a primarily preventive bivalent recombinant human hookworm vaccine,

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Table 1.1 Poverty in the Americas. (Data from [1, 4])

	Region	
	Latin America & Caribbean	United States
Population in 2010	578 million	306 million
Living on less than $1 per day in 2005	8.2 %	
Estimated number of people living on less than $1 per day	48 million	
Living on less than $2 per day in 2005	17.1 %	
Estimated number of people living on less than $2 per day	99 million	
Living below the poverty line in 2010		15.1 %
Number of people living below the poverty line		46 million

Table 1.2 Countries in the Americas with the lowest Human Development Indices. (Data from [3])

Country	2008 HDI rank	Comparator countries with similar HDI rank
Haiti	148	Sudan, Kenya
Guatemala	121	South Africa, Gabon
Nicaragua	120	South Africa, Gabon
Honduras	117	Mongolia, Kyrgyzstan
Bolivia	111	Egypt, Indonesia
Guyana	110	Egypt, Indonesia
El Salvador	101	Algeria, Cape Verde, Vietnam
Paraguay	98	Sri Lanka, Iran

which is about to enter phase 1 clinical testing in Brazil, as well as a new therapeutic Chagas disease vaccine in collaboration with several Mexican institutions. The Chagas disease vaccine would be administered to seropositive patients to delay or prevent the onset of Chagasic cardiomyopathy (secondary prevention). Together, MDA and the development of new anti-poverty vaccines afford an opportunity to implement effective control and elimination strategies for the major NTDs in the Americas.

1.1 Introduction: Poverty in the Americas

Today, almost one-fifth of the 578 million people who live in the Latin American and Caribbean (LAC) region live in severe poverty. As shown in Table 1.1, an estimated 50 and 100 million people live on less than US$1.25 per day (the World Bank poverty level) and US$2 per day, respectively [1]. These populations represent the LAC region's bottom 100 million. Poverty has an uneven distribution in the LAC region, such that the bottom 100 million cluster in defined geographical areas of Mexico and Central America, South America, and in areas with large indigenous populations. For example,

almost one-half of rural households in Mexico are considered poor, most of them in southern Mexico; one-half of the population of Central America also lives in poverty [2]. Four of the eight LAC countries with the lowest human development indices (HDIs) are found in Central America, including Guatemala, Nicaragua, Honduras, and El Salvador (Table 1.2) [3], where poverty disproportionately affects indigenous populations [2]. Among South American populations, there are an estimated 20 million poor in Brazil, particularly in the northeastern part of the country, and especially among women and children [2]. Some of the poorest people in LAC live in indigenous communities and mountainous areas of Bolivia, Peru, and Ecuador and in the Chaco of Bolivia and Paraguay [2, 3]. More than one-third of impoverished LAC populations live in the Andean region [2]. The Caribbean countries of Haiti and Guyana also stand out for their low HDIs [3]. Overall, the HDIs of the poorest countries in the LAC region are similar to many poor African and Asian countries (Table 1.2).

Poverty in the Americas is not restricted to the LAC region [4–6]. With the recent recession in the US the percentage of people living below the poverty line has increased to its highest level in more than 50 years [4]. It was recently determined that the US poverty rate has increased to 15 %, with 46 million people living below the poverty line [4]. Poverty in the US also has an uneven distribution, with the highest rates along the Gulf Coast and in South Texas [5, 6]. Poverty

Table 1.3 The neglected tropical diseases of the Latin American and Caribbean (LAC) region. (Modified from [9])

Disease	Estimated DALYs in LAC (million)	Estimated number of cases (million)	Estimated bottom 100 million affected (%)	Countries most affected
Hookworm	0.1–1.9	50	50	Brazil, Paraguay, Guatemala, Colombia
Ascariasis	0.1–1.1	84	84	Brazil, Mexico, Guatemala, Argentina
Trichuriasis	0.3–1.1	100	100	Brazil, Mexico, Colombia, Guatemala
Chagas disease	0.7	8–9	10	Bolivia, Mexico, Colombia, Central America
Dengue	0.1	>0.6	1	Brazil, Caribbean
Leishmaniasis	<0.1	0.1	1	Brazil, Colombia, Peru, Nicaragua, Bolivia
Schistosomiasis	<0.1	1.8	2	Brazil, Venezuela, Suriname, Saint Lucia
Lymphatic FIlariasis	<0.1	0.7	1	Haiti, Brazil, Dominican Republic, Guyana
Trachoma	<0.1	1.1	1	Brazil, Guatemala
Leprosy	<0.1	<0.1[a]	<1	Brazil
Onchocerciasis	<0.1	<0.1	<1	Guatemala, Mexico, Ecuador, Venezuela, Brazil, Colombia
Human rabies from dogs	<0.1	<100[b]	<1	Haiti, Bolivia
Cysticercosis	ND	0.4	<1	ND
Toxoplasmosis	ND	ND	ND	Brazil

ND not determined

[a] New cases

[b] Cases

1.2 Neglected Tropical Diseases in the Americas

Among the LAC region's bottom 100 million, approximately one-half lack adequate food [8]. Equally tragic are the results of our previous analysis showing that almost all the bottom 100 million suffers from one or more neglected tropical disease [9]. Globally, the neglected tropical diseases or 'NTDs' comprise 17 major chronic parasitic and related infections [10–12]. Importantly, they not only adversely affect the health of infected individuals, but also trap people in poverty, because of their long-term impact on child growth and cognitive development, pregnancy outcome, and occupational productivity [11, 13]. Shown in Table 1.3 is a ranking of the leading NTDs in the LAC region by disease burden, measured in disability-adjusted life years.

also occurs in the Canadian and the Alaskan Arctic among Inuit and related populations [7].

The leaders are hookworm and other soil-transmitted helminths, Chagas disease (American trypanosomiasis), and dengue, followed by leishmaniasis, schistosomiasis, lymphatic filariasis, trachoma, leprosy, onchocerciasis, and cysticercosis [9]. Almost all of the bottom 100 million are infected with one or more soil-transmitted helminths, including trichuriasis, ascariasis, and hookworm. Approximately 10 % are affected by Chagas disease and 1 % by dengue fever. Precise prevalence data for toxoplasmosis in the LAC region is lacking, however it is believed to be common, especially in Brazil [14]. With the exception of Chagas disease and possibly trachoma, most of the NTDs were introduced into the Americas through the Atlantic slave trade [15], and these conditions still disproportionately affect African-American and indigenous populations [9].

Table 1.3 also shows the disproportionate representation of the most common NTDs in low HDI countries, for example, hookworm in Paraguay and Guatemala, and Chagas disease in Bolivia and in several Central American countries

Table 1.4 Major neglected infections of poverty in the US. (Modified from [5])

Disease	Estimated number of cases (million)	Major populations affected
Toxocariasis	1–3	African American
Trichomoniasis	0.9	African American
Chagas disease	Up to 1	Hispanic American
Dengue	0.1–0.2	Hispanic American
Cysticercosis	0.04–0.16	Hispanic American

[9]. Dengue and dengue haemorrhagic fever (DHF) have become more prevlent throughout the LAC region [16]. Brazil has the largest number of NTDs of any country in the LAC region [9, 17], mostly among impoverished populations in the northeastern part of the country. However, the severe poverty in northeastern Brazil does not affect its HDI sufficiently to rank that country in the bottom tier.

The economic downturn in the US has increased media attention to the plight of the Northern American poor, and with it, increased recognition of a hidden underbelly of neglected infections of poverty [5, 6]. Some NTDs found in the LAC region are also found in South Texas and the Gulf Coast, including Chagas disease, cysticercosis, and dengue (Table 1.4) [5, 6]. Of particular concern is evidence suggesting autochthonous transmission of Chagas disease and dengue in these regions. High prevalence rates of toxocariasis and trichomoniasis also occur among people living in poverty in the US and presumably throughout the Americas [5, 6].

1.3 Adverse Effects on Maternal and Child Health, Poverty, and Conflict

Overall, there are three major adverse health and socioeconomic consequences of high prevalence NTDs in the Americas, related to their impact on (1) maternal and child health, (2) poverty, and (3) conflict (Box 1).

Box 1 Effects of the High Burden NTDs on Maternal and Child Health, Poverty, and Conflict in the LAC Region

Maternal Child Health
- Hookworm is associated with anemia in children and pregnancy leading to intellectual and cognitive deficits, low neonatal birth weight, and increased maternal morbidity.
- Chagas disease in pregnancy increases the risk of miscarriage and preterm birth and is associated with vertical transmission in 1 in 20 seropositive mothers resulting in neonatal infection and death.
- Dengue and DHF disproportionately affect children and in pregnancy are linked with elevated rates of preterm birth, cesarean delivery, and vertical transmission.

Poverty
- Hookworm is associated with 40 % reduction in future wage earning.
- Chagas disease results in economic losses exceeding US$ 1 billion annually with lifetime costs per patient averaging almost US$ 12,000.
- High dengue economic costs remain under investigation.

Conflict
- Destabilizing effects on agricultural productivity, land use, food security, economic growth.
- Links with guerilla movements and narcotrafficking.

1.3.1 Maternal and Child Health

It is not commonly appreciated that the highest burden NTDs in the LAC region, including hookworm and the other soil-transmitted hel-

minths, Chagas disease, and dengue exert special adverse consequences for the health of children and women.

Hookworms cause chronic intestinal blood loss leading to iron deficiency anemia and protein malnutrition. Hookworm anemia is more pronounced in women and children because of their unique iron metabolisms [18]. Throughout the LAC region *Necator americanus* is the predominant species causing hookworm disease and anemia [9, 18]. In children this infection is associated with deficiencies in physical growth and cognitive development, while in pregnancy it is linked to increased maternal morbidity and poor neonatal outcomes [18]. Two recent systematic reviews confirm the association between hookworm infection and anemia in children and pregnant women [19, 20]. Specifically, in the LAC region *N. americanus* infection was recently linked to anemia in young children in rural Minas Gerais State, Brazil [21], and with low neonatal birth weight among pregnant women in Peru [22].

Chagas disease has also emerged as an important maternal and child health problem, with mother-to-child transmission increasingly recognized as an important route of infection [23]. Pregnancy has been shown to enhance *T. cruzi* parasitemia [24], with vertical transmission and congenital infection (characterized by hepatosplenomegaly, hydrops, and neonatal death) occurring in an estimated 1 in 20 seropositive mothers [23, 25]. In North America alone there are an estimated 40,000 pregnant women with Chagas disease, and several thousand newborns are likely to be infected with *T. cruzi* annually [26]. The numbers throughout Latin America would therefore be expected to be several-fold higher. Aside from congenital infection, Chagas disease during pregnancy increases the risk of miscarriage, preterm birth, and neonatal infection, which may cause infant death or severe sequelae [25].

Dengue and DHF disproportionately affect children in much of the developing world, including during outbreaks in the LAC region [27, 28]. Additionally, a recent systematic review confirms the harmful effects of dengue during pregnancy, including elevated rates of preterm birth, Cesarean delivery, and vertical transmission [29].

1.3.2 Poverty and Conflict

The mechanisms by which the NTDs actually promote or cause poverty have been reviewed previously. These mechanisms include specific effects on maternal and child health, in addition to the health of workers in the tropics, and agricultural productivity [11, 13]. A retrospective analysis of hookworm in the American South revealed that chronic hookworm infection in childhood reduce future wage earning by 40 % [30], postulated to be due to its harmful effects on child cognitive development. Similarly, Chagas disease is associated with US$1.2 billion in economic losses annually, because of its impact on maternal and child health and occupational productivity, as well as the very high costs of treatment, with a lifetime cost averaging almost US$12,000 per patient [31]. Economic losses from dengue in India were recently estimated to be US$27.4 million during a 2006 epidemic [32], however a recent systematic review concluded that the economic literature on dengue is "sparse" and results are conflicting because of the use of inconsistent assumptions [33]. NTDs also promote conflict and warfare through destabilizing effects on agricultural productivity, abandonment of arable lands, food insecurity and decrements in educational attainment and wage earning [34]. In the LAC region an association has been noted between guerilla movements and the trafficking of narcotics with Chagas disease and leishmaniasis [35].

1.4 Eliminating NTDs in the Americas

Several high-prevalence NTDs, including lymphatic filariasis, onchocerciasis, and trachoma are being eliminated as public health problems in the LAC region [9]. The term elimination refers to reduction in the incidence of a specific infection to zero or below a threshold that can no longer sustain transmission. Such elimination efforts rely heavily on mass MDA, often with drugs donated by the pharmaceutical industry, including ivermectin for onchocerciasis; diethyl-

Table 1.5 Latin American and Caribbean countries that have successfully eliminated their neglected tropical diseases as a public health problem. (Modified from [38])

Disease	Countries
Chagas disease	Brazil, Chile, Uruguay
Schistosomiasis	Dominican Republic, Guadeloupe, Martinique, Puerto Rico
Lymphatic Filariasis	Costa Rica, Suriname, Trinidad and Tobago
Trachoma	Mexico
Leprosy	All except Brazil
Onchocerciasis	Elimination pending in the six affected countries

carbamazine and albendazole for lymphatic filariasis; and azithromycin for trachoma [36]. Through MDA along with other control measures including integrated vector management, lymphatic filariasis has been eliminated from Costa Rica, Suriname, Trinidad, and Tobago (Table 1.5), with expectations that this disease might also be eliminated from the few remaining endemic countries, including Brazil, Haiti, Dominican Republic, and Guyana by 2020.

Similarly through the Onchocerciasis Elimination Programme of the Americas (OEPA) it should be possible to eliminate onchocerciasis in the six remaining endemic countries, Guatemala, Mexico, Venezuela, Ecuador, Colombia, and Brazil, where approximately 0.52 million people are at risk for infection, while trachoma remains only in Brazil, Guatemala, and in five municipalities of neighboring Chiapas state in Mexico [37–39]. Today, such elimination efforts are being coordinated by the Pan American Health Organization in association with the Interamerican Development Bank and a Global Network for NTDs based at the Sabin Vaccine Institute. It has been estimated that US$128 million will be required to eliminate these three infections in the LAC region by the year 2020 [39], and a special "LAC fund" has been established in order to receive public and private donations for this purpose.

Through MDA with praziquantel, schistosomiasis remains endemic in only four LAC countries, including Brazil, St. Lucia, Suriname, and Venezuela, having been previously eliminated from Martinique, Guadeloupe, Puerto Rico and the Dominican Republic, with an expectation that this disease might be eliminated from the Carib-

bean in the coming years [37, 38]. Because of multi-drug therapy with dapsone, clofazimine, and rifampin, fewer than 34,000 registered cases of leprosy remain in the Americas, with all but about 4,000 cases being in Brazil [40]. Canine rabies has also been largely eliminated. The majority of the cases of canine rabies transmitted to humans occur in poor neighborhoods in Haiti and Bolivia [37].

The highest prevalence NTDs, including hookworm and the other soil-transmitted helminths, Chagas disease, dengue, and leishmaniasis, in addition to schistosomiasis in Brazil, will not be eliminated solely by relying on existing technologies, even though some progress has been achieved with MDA with albendazole/mebendazole and praziquantel (for soil-transmitted helminths and schistosomiasis, respectively) and integrated vector management for Chagas disease, dengue, and leishmaniasis [38]. In the case of hookworm, high rates of mebendazole drug failure and post-treatment reinfection have been reported, and although repeated MDA targeting children may have an impact on reducing the transmission of ascariasis and trichuriasis, it is not expected to have an impact on hookworm infection because of hookworm's unique transmission dynamics among adults [41]. Similarly, integrated vector management has so far been successful for eliminating Chagas disease only in the southern Cone of South America (Brazil, Chile, Uruguay), whereas elsewhere insecticide resistance, reinvasion and recolonization of reduviid vectors after spraying have thwarted control efforts [42]. Therefore, a recent "audacious" call to eliminate all of the 17 NTDs as defined by the WHO will require a new generation of technologies, especially the development of NTD vaccines [38].

1.5 New 'Anti-Poverty Vaccines' will be Required

Vaccines to combat the NTD vaccines are also known as the 'anti-poverty vaccines' because of their potential impact on economic development in addition to improving health [13, 43].

Table 1.6 Vaccines under development for Latin America's neglected tropical diseases. (Modified from [43])

Disease	Type of vaccine under development	Stage of development	Organization leading vaccine development efforts	Industrial partners
Chagas disease	Human therapeutic vaccine	Preclinical	Sabin Vaccine Institute PDP (Chagas Vaccine Initiative)	Birmex and CINVESTAV
Cysticercosis	Veterinary vaccine	Animal trials	Universidad Nacional Autonoma de Mexico	Pending
Dengue	Human preventive vaccine	Phase 1 and 2	GSK, Merck & Co., Sanofi-Pasteur, Instituto Butanan	GSK, Merck & Co., Sanofi-Pasteur, Instituto Butanan
Foodborne trematode infections	Veterinary vaccine	Animal trials	FIOCRUZ (Oswaldo Cruz Foundation)	Ouro Fino
Hookworm infection	Human preventive vaccine	Phase 1	Sabin Vaccine Institute PDP (Human Hookworm Vaccine Initiative)	FIOCRUZ-Bio-manguinhos; Aeras, Fraunhofer CMB
Leishmaniasis	Human preventive, therapeutic, and veterinary	Phase 1 and 2 and animal trials	Infectious Diseases Research Institute (IDRI)	Instituto Butantan
Schistosomiasis	Human preventive vaccine	cGMP manufacture[*]	Sabin Vaccine Institute PDP (Schistosomiasis Vaccine Initiative), FIOCRUZ	Aeras, Instituto Butantan, Ouro Fino

[*]*cGMP* current good manufacturing practices

Table 1.6 lists the major anti-poverty vaccines under development in the Americas, which include new human vaccines against *Trypanosoma cruzi*, dengue virus, hookworm, leishmania species, and schistosoma species, in addition to veterinary vaccines against *Taenia solium* and *Fasciola hepatica* (a food-borne trematode) to prevent transmission to humans [43]. Of these vaccines, only the dengue vaccine is being developed and produced by the multinational vaccine manufacturers, including three vaccines being developed independently by Merck & Co., GlaxoSmithKline, and Sanofi-Pasteur [43, 44]. The other anti-poverty vaccines are largely being developed by non-profit product development partnerships (PDPs) in association with developing country manufacturers [43]. One reason why dengue vaccine development receives investment from multinational pharmaceutical companies is the large potential commercial market for such a product, given that dengue affects people living in wealthier urban centers, whereas the other anti-poverty vaccines target almost exclusively the bottom 100 million in the LAC region, and their counterparts in Africa and Asia [43]. There is some overlap, however, as a Brazilian company Ouro Fino Animal Health is developing a *Fasciola hepatica* vaccine for livestock (http://www.veterinaryproducts1.com/supplier/ouro-fino-animal-health.html), which could prevent transmission to humans, while the developing country manufacturer, Instituto Butantan is also developing its own dengue vaccine (http://www.butantan.gov.br/home).

Today, efforts to develop and test anti-poverty vaccines targeted for human disease in the Americas are being led by PDPs, in collaboration with Latin American developing country manufacturers [43]. Most of these manufacturers, in turn, are owned and operated by scientific enterprises directly supported by federal and state governments in Latin America, including FIOCRUZ Bio-Manguinhos (through support of the Brazilian Ministry of Health), Instituto Butantan (State of Sao Paulo, Brazil), Birmex (Laboratorios de Biologicos y Reactivos de Mexico, Mexican Ministry of Health), and Cuba's Instituto Finlay [43]. Some examples of PDP-manufacture collaborations are shown in Table 1.6 and include the non-profit Infectious Disease Research Institute (Seattle, Washington), which is working with Instituto Butantan for leishmaniasis vaccine development, while the Sabin Vaccine Institute PDP (Houston, Texas and Washington, DC) is working with FIOCRUZ, (together with the US-based Aeras and Fraunhofer Center for

Molecular Biolotechnology) to develop a human hookworm vaccine, and with CINVESTAV (Centro de Investigacion y de Estudios Avanzados del Instituto Politecnico Nacional (Center for Research and Advanced Studies)) and Birmex for a Chagas disease vaccine. Each of these vaccines is either at the stage of pilot manufacture under cGMP (current good manufacturing practices) or in early clinical development [43].

1.6 Hookworm and Chagas Disease Vaccines

Progress in the development of a vaccine against hookworm was reviewed recently [45]. In brief, the human hookworm vaccine is a bivalent vaccine comprised of two recombinant antigens, which are parasitic enzymes involved in blood feeding [45]. The vaccine targets the adult stages of *N. americanus*, the most common hookworm worldwide and almost the exclusive hookworm in the LAC region. One of the antigens is a recombinant *N. americanus* glutathione S-transferase (*Na*-GST-1) expressed in yeast, an enzyme required by the parasite for heme binding and heme detoxification. The other is a recombinant *N. americanus* aspartic protease (*Na*-APR-1) expressed in plants, an enzyme required for hemoglobin degradation. Both recombinant antigens induce high levels of IgG antibody and have demonstrated protective immunity in laboratory animals, with reduced host worm burden and/ or blood loss [45]. The recombinant proteins have completed pilot cGMP manufacture, and *Na*-GST-1 has entered phase 1 clinical testing. Ultimately both antigens would be formulated on alum and possibly combined with a second adjuvant such as a synthetic lipid A [45]. The target product profile of the human hookworm vaccine relies on its use to prevent moderate and heavy *N. americanus* infections in children under the age of 10 years [45]. The vaccine may be incorporated into the Expanded Program on Immunization (EPI) in order to be co-administered with measles and other childhood vaccines to infants (children under the age of one), or it may be co-administered with a single dose of anthelmin-

thic drug in older children already infected with hookworm. The desired efficacy of the human hookworm vaccine is at least 80 % against moderate and heavy hookworm infections for at least 5 years after immunization [45]. The cost-effectiveness of such a vaccine was recently confirmed under a number of different scenarios [46].

Progress on the development of a vaccine for Chagas disease has also been reviewed recently [47]. Unlike the human hookworm vaccine, which is a primarily preventive vaccine, the Chagas vaccine is being proposed as a therapeutic vaccine for the treatment of individuals who have been infected with *T. cruzi* and have seroconverted. In such individuals, who have so-called "indeterminate" status (with no clinical, electrocardiographical or radiological evidence of disease), approximately 20–30 % subsequently develop Chagasic cardiomyopathy [48]. There is an urgent need for new therapeutic approaches for these patients, especially in order to eradicate *T. cruzi* parasites in the myocardium that are responsible for progression to cardiomyopathy and heart failure. A recent meta-analysis of the two currently available drugs benznidazole and nifurtimox concluded that their efficacy in late chronic infection is doubtful and does not result in seroreversion [49, 50], although a larger randomized placebo-controlled study is in progress [51]. Moreover, prolonged treatment courses lasting 2–3 months are required and result in serious side effects in up to one-half of the patients, with 10–20 % discontinuing therapy as a result [52, 53]. The drugs are also contraindicated in pregnancy and are extremely expensive to purchase and administer [54].

The target groups for this therapeutic vaccine are adults, particularly pregnant women to improve birth outcomes and prevent congenital infection, and children over the age of two, in Chagas disease-endemic areas. The vaccine is under development by the Sabin Vaccine Institute PDP and the Texas Children's Center for Vaccine Development in collaboration with the US National Institutes of Health and three Mexican institutions, the Autonomous University of Yucatan (UADY), CINVESTAV, and Birmex (Mexico's public sector vaccine manufacturer).

The bivalent vaccine is comprised of two *T. cruzi* recombinant proteins formulated on alum. One of the antigens is a unique *T. cruzi* 24 kDa antigen (Tc24) and the other belongs to a family of *T. cruzi* surface transialidases (TSA-1). Proof of concept for the protective effect of these antigens is based on experimental immunizations in *T. cruzi*-infected laboratory animals, together with identifiable mechanisms of protective immunity [47, 55–58]. In acutely and chronically-infected mice the combined antigens produced significantly reduced parasitemia and myocardial inflammation compared to controls [58]. Because protection requires the stimulation of CD8+ T cells and production of interferon gamma [58], the vaccine incorporates a second adjuvant comprised of a synthetic lipid A. The requirement for this second adjuvant will be determined pending preclinical studies and early clinical trials.

Ascertaining the feasibility of expression of Tc24 and TSA-1 as soluble recombinant proteins for the purpose of process development, scale-up and current Good Manufacturing Practices (cGMP) manufacture is currently in progress. It is expected that during process development and scale-up, studies to evaluate protein attributes and stability will be established. In addition, formulation studies with alum and other adjuvants will be performed. Ultimately, the ability of these formulations to protect mice from acute and chronic *T. cruzi* infections will be confirmed.

It is anticipated that successfully eliciting Th1 immune responses will be a key to human therapeutic vaccination against Chagas disease [47, 55–58]. Th1-type immune responses are characterized by the generation of CD8+ T cells, which can target intracellular pathogens [55–58]. While several purified recombinant protein vaccines are in clinical development, they are limited by poor immunogenicity and inadequate stimulation of Th1 immunity [59, 60]. Particulate-based systems can increase the delivery of antigens to antigen-presenting dendritic cells, while simultaneously maintaining antigen integrity [60]. Equally important, particulate systems can co-deliver immunopotentiating agents and activate CD8+ cells. Using this strategy, the two recombinant *T. cruzi* antigens under development will be encapsulated in nanoparticles that will also contain an adjuvant molecule such as the TLR3 agonist poly(I:C). It has been previously shown that ovalbumin formulated in this manner elicits antigen-specific CD8+ T cell responses *in vitro* that greatly exceed those produced either by the antigen alone or antigen encapsulated without the TLR3 agonist [61]. We will therefore attempt to simultaneously develop the Chagas disease vaccine as a nanoparticle vaccine using these technologies, and evaluate it in an experimental therapeutic mouse model for *T. cruzi* infection. An alternate delivery system under consideration is a viscous polysaccharide solution, which forms an extracellular antigen depot at the injection site [62]. Still another option is to examine viral vectors and heterologous prime-boost approaches. This approach has been successful for other systems requiring induction of Th1-type immunity [63–65].

1.7 Concluding Statement

While estimates indicate that less than US$200 million will be required to eliminate lymphatic filariasis, onchocerciasis, and trachoma from the Americas, there are still the added costs of continuing control efforts with MDA for other NTDs (with costs possibly exceeding US$300 million) [37], in addition to the costs of integrated vector management and the estimated US$1.2 billion required annually for Chagas disease alone, presumably much of which is treatment costs [66] In addition to jumping the scientific hurdles, the challenge of vaccine development includes demonstration of cost effectiveness, and this has now been shown for both human hookworm and Chagas disease vaccines [46, 66]. These two vaccines, in addition to vaccines to prevent leishmaniasis, schistosomiasis, and dengue, represent urgently needed control measures for a full scale elimination effort for all of the major NTDs in the Americas. Such activities in the Western Hemisphere are part of a larger "audacious goal" for

elimination of all of the 17 NTDs as the most common infections of the world's poor and legacies of neglect, ignorance, and slavery [38]. Thus, an opportunity is now in hand for the major PDPs, research institutes and developing country manufacturers in the LAC region, together with the major development banks and PAHO to draft an elimination strategy for the NTDs in the Americas.

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Non-typhoidal Salmonella in Children: Microbiology, Epidemiology and Treatment

2

Gabrielle M. Haeusler and Nigel Curtis

Abstract

Non-typhoidal *Salmonellae* (NTS) are an important cause of infectious diarrhoea world-wide. In the absence of immune deficiency, gastroenteritis caused by NTS is usually mild, self limiting and rarely requires intervention. NTS are also an important cause of invasive disease, particularly in developing countries, likely secondary to the high prevalence of co-existing malnutrition, malaria and HIV infection. This review provides an overview of the microbiology, epidemiology and pathogenesis of NTS, and compares recommendations for the treatment of NTS gastroenteritis in children.

2.1 Introduction

Non-typhoidal *Salmonellae* (NTS) are an important cause of infectious diarrhoea world-wide. In the absence of immune deficiency, gastroenteritis caused by NTS is usually mild, self limiting and rarely requires intervention. NTS are also an important cause of invasive disease, particularly in developing countries, likely secondary to the high prevalence of co-existing malnutrition, malaria and HIV infection. Antibiotic treatment of NTS gastroenteritis has been the subject of a meta-analysis, but questions regarding exactly which patients should be treated and the optimal regimen remain unanswered. This review provides an overview of the microbiology, epidemiology and pathogenesis of NTS, and compares recommendations for the treatment of NTS gastroenteritis in children.

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2.2 Classification and Microbiology

The genus *Salmonella* belongs to the family of Enterobacteraciae. *Salmonella* are separated into two species, *Salmonella enterica* and *Salmonella bongori* (previously classified as subsp. V.), with the former being further classified into six subspecies (I, *S. enterica* subsp. *enterica*; II,

Table 2.1 Number of serotypes in each subspecies. (Based on data from Grimont et al. [1])

Species	Subspecies	Number of serotypes
Salmonella enterica	*enterica* (I)	1,531
	salamae (II)	505
	arizonae (IIIa)	99
	diarizonae (IIIb)	336
	houtenae (IV)	73
	indica (VI)	13
Salmonella bongori (V)	–	22
Total		2,579

Table 2.2 Examples of clinically significant *Salmonella* serotypes and relevant clinical syndromes according to serogroup. (Based on data from Grimont et al. [1])

Serogroup	Serotype example[a]	Clinical syndrome
A	Paratyphi A	Enteric fever
B	Paratyphi B	Enteric fever
	Typhimurium	NTS
	Heidelberg	NTS
C1	Paratyphi C	Enteric fever
	Choleraesuis	NTS
	Virchow	NTS
C2	Newport	NTS
D1	Typhi	Enteric fever
	Enteritidis	NTS
	Dublin	NTS

[a] All example serotypes are members of subspecies *Salmonella enterica* subspecies *enterica*

S. enterica subsp. *salamae*; IIIa, *S. enterica* subsp. *arizonae*; IIIb, *S. enterica* subsp. *diarizonae*; IV, *S. enterica* subsp. *houtenae*; and VI, *S. enterica* subsp. *indica*) [1]. While an alternative nomenclature describes the genus as a single species, *Salmonella choleraesuis*, the Judicial Commission of the International Committee on the Systematics of the Prokaryotes supports the two-species designation [2].

Salmonellae are motile, Gram negative, facultative anaerobic bacilli which rarely ferment lactose [3]. Within the seven subspecies, more than 2,500 serotypes (or serovars) have been reported (Table 2.1) [1]. Salmonellae are classified according to antigenically diverse surface antigens: polysaccharide O (somatic) antigens, H (flagellar) antigens and Vi (capsular) antigens [3]. Agglutination reactions based on the O-antigen are used by most clinical laboratories to divide Salmonella into serogroups which include, but are not limited to, A, B, C1, C2, D and E (Table 2.2) [1]. Strains in these six serogroups cause most of the NTS infections in humans [4]. Formal serotyping, usually by reference laboratories, is required to differentiate clinically significant serotypes as cross-reactivity occurs. A detailed list of all currently recognised *Salmonella* serotypes is available elsewhere [4].

Salmonella nomenclature and syntax is potentially confusing. An example of a correct *Salmonella* subspecies and serotype designation is *Salmonella enterica* subspecies *enterica* serotype Typhimurium. An accepted abbreviation of this full taxonomic designation is *Salmonella* ser.

Typhimurium (capitalised and not italicised) at the first citation and subsequently *Salmonella* Typhimurium [5].

Salmonella enterica subspecies *enterica* contains almost all the serotypes pathogenic for humans [3]. Although many *Salmonella* serotypes exist, they can be broadly categorised as typhoidal or non-typhoidal *Salmonella* (NTS) depending on the clinical syndrome with which they are predominantly associated (Table 2.2). The typhoidal Salmonella include the *S. enterica* subspecies *enterica* serotypes Typhi and Paratyphi A-C and typically cause systemic illness with little or no diarrhoea. The much larger group of NTS primarily induce acute, self-limiting gastroenteritis and is the focus of this review.

2.3 Epidemiology

In contrast to *Salmonella* Typhi and *Salmonella* Paratyphi, which are rarely encountered outside endemic countries or in returned travellers, NTS have a worldwide distribution. While the true incidence is unknown, there are an estimated 93.8 million episodes and 155,000 deaths each year attributable to NTS [6]. Data from the World Health Organization (WHO) Global Foodborne Infections Network (GFN) indicate that

Salmonella Typhimurium and *Salmonella* ser. Enteritidis account for nearly 80 % of all human isolates reported globally [7].

Unlike *Salmonella* Typhi and *Salmonella* Paratyphi, which have host specificity for humans, NTS can be acquired from both animal and humans. While poultry and eggs remain the most common source of NTS, other animal reservoirs include reptiles, rodents, cats and dogs [8, 9]. A case control study in the United States found that up to 6 % of all sporadic NTS infections are attributable to reptile or amphibian contact [10].

Transmission is predominantly foodborne, although other modes include consumption of contaminated water, contact with infected animals and nosocomial exposure [4, 11]. The incubation for NTS gastroenteritis depends on the host and the inoculum. It is usually 12–36 h, although incubation periods of up to nearly 2 weeks have been reported with certain strains [12–14].

2.4 Pathogenesis

Salmonellae are facultative intracellular pathogens that can survive within host macrophages [15]. Unlike typhoidal salmonella, which have the ability to evade the immune system, NTS tend to induce a localised inflammatory response in immunocompetent individuals, provoking a large influx of polymorphonuclear leukoytes to the intestinal lumen [16]. They can also colonise small and large intestinal mucosa thus facilitating prolonged periods of shedding [16].

Host factors predisposing to severe NTS infection include reduced gastric acidity, impaired cell mediated and humoral immunity, and impaired phagocytic function [16–18]. Salmonellae are unable to survive at a gastric pH less than 2.5 [19] and patients with anatomical or functional achlorhydria are at increased risk of developing infection [20]. This is especially relevant to neonates where the combination of relative achlorhydria and frequent milk feeds may contribute to their increased risk of NTS bacteraemia [21].

T-cell immunity is important in controlling *Salmonella* as evidenced by increased suscep-

tibility to invasive NTS in HIV-infection [22, 23] and with corticosteroid use [24]. Children with congenital defects in humoral immunity including X-linked agammaglobulinaemia and common variable immunodeficiency, are also reported to have increased risk of persistent diarrhoea and invasive disease [16]. Impaired phagocytic function seen in chronic granulomatous disease, haemoglobinopathies and malaria similarly increase the risk of invasive NTS infection [16]. In addition, co-infection with *Schistosoma* has been reported to cause prolonged and severe illness due to altered macrophage function and replication and survival of *Salmonella* within the parasite [25].

2.5 Clinical Syndromes

Clinical manifestations of NTS can be broadly divided into four groups:
1. Acute gastroenteritis
2. Extra-intestinal infection
3. Non-infectious sequelae
4. Salmonella carriage

2.5.1 Acute Gastroenteritis

Non-typhoidal salmonellae usually cause an acute self-limiting gastroenteritis. In contrast to typhoidal salmonella, infection with NTS results in diarrhoea. This is profuse and usually non-bloody. Associated symptoms of fever, abdominal cramping, nausea and vomiting may also occur [4]. Diagnosis is confirmed on stool culture. Fluid and electrolyte disturbances are the most frequent complication of NTS gastroenteritis. Asymptomatic gastrointestinal infection can also occur; however, given the rate of convalescent NTS excretion following acute infection, the true incidence is unknown [26].

2.5.2 Extra-intestinal Infection

Overall, NTS bacteraemia is reported in up to 9 % of patients with acute gastroenteritis [27].

Choleraesuis, Heidelberg and Dublin are among some of the serotypes more frequently associated with bacteraemia [28]. The incidence of invasive disease is also modified by factors such as age, region and underlying immune status. Surveillance data from the United States showed the incidence of invasive NTS was 7.8 cases per 100,000 in infants (aged less 1 year) compared to less than 0.8 cases per 100,000 in older children [29]. Significant discordance in the burden of invasive NTS between continents exists with an estimated annual incidence of up to 388 per 100,000 children (aged less 5 years) in Africa [30]. In this continent, the common invasive serotypes are Typhimurium and Enteritidis. The high prevalence of malaria in Africa, and its association with invasive NTS, has been postulated as one reason for this difference [31]. Interestingly, recent studies in the Gambia, Kenya and Tanzania have shown that the marked decline in malaria prevalence has been paralleled by a similar reduction in invasive NTS [32].

Bacteraemia may result in focal NTS infection at any site, including the central nervous system [33, 34]. Risk factors for focal disease are similar to those for bacteraemia [35], although focal infections in non-immunocompromised children are well described [36]. In one study, 7 of 12 (58 %) immunocompromised children developed focal infection compared with 5 of 132 (4 %) non-immunocompromised children [37].

Salmonella bacteraemia usually presents with fever and/or sepsis. Persistent bacteraemia has been documented in afebrile, well-appearing infants with NTS gastroenteritis emphasising the importance of blood cultures in this population [38]. Conversely, bacteraemia has been described in immunosuppressed patients without a history of gastroenteritis [24]. Notably, in the African setting, the majority of invasive NTS cases do not have gastroenteritis.

Salmonella can be cultured from standard blood culture media [3]. Positive urine culture with *Salmonella spp*. may indicate bacteraemia rather than faecal contamination [39]. Similar to *Salmonella* Typhi, culture of bone marrow samples may increase the diagnostic yield of NTS [30, 40].

2.5.3 Non-infectious Sequelae

Non infectious sequelae following infectious gastroenteritis are well described [41]. Reactive arthritis after NTS infection has been reported in up to 29 % of patients [42]. There are conflicting data on the association between antibiotic treatment for acute NTS gastroenteritis and subsequent development of musculoskeletal symptoms, some suggesting a decreased risk and others an increased risk [42].

2.5.4 Salmonella Carriage

Salmonella carriage is defined as asymptomatic excretion following acute infection and can be divided into convalescent carriage and chronic carriage.

2.5.4.1 Convalescent Carriage
Convalescent carriage occurs frequently after symptomatic or asymptomatic NTS infection. Fig. 2.1 shows the duration of excretion of NTS in 13 studies according to age [43]. The median duration of NTS excretion in children less than 5 years of age is 7 weeks, with 18 % remaining culture positive at 6 months. Carriage is shorter in older children with a median duration of 3–4 weeks with only 0.3 % remaining culture positive at 6 months. In addition to younger age, factors associated with prolonged duration of excretion include symptomatic infection, treatment with antibiotics and infection with strains other than *Salmonella* Typhimurium (Fig. 2.2) [43]. Episodic excretion is not uncommon.

2.5.4.2 Chronic Carriage
Documented excretion of NTS for more than 1 year is defined as chronic carriage [44]. This occurs in up to 2.6 % of children under 5 years of age and 0.3 % of older children [43].

Fig. 2.1 Duration of excretion of NTS by age (by permission of Oxford University Press). [43]
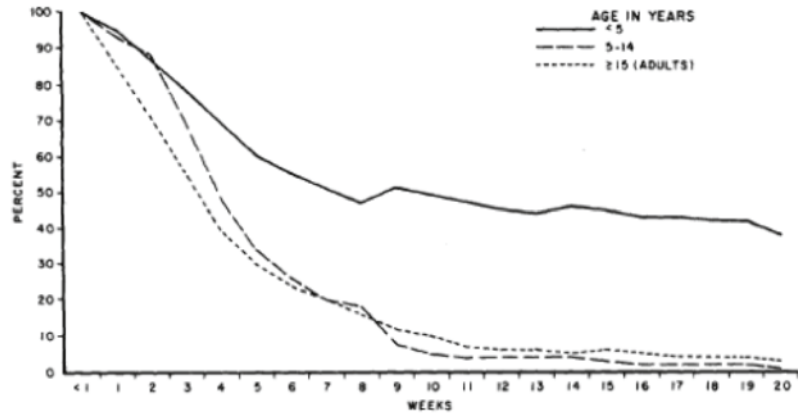
Fig. 2.2 Duration of excretion of NTS by serotype (by permission of Oxford University Press). [43]
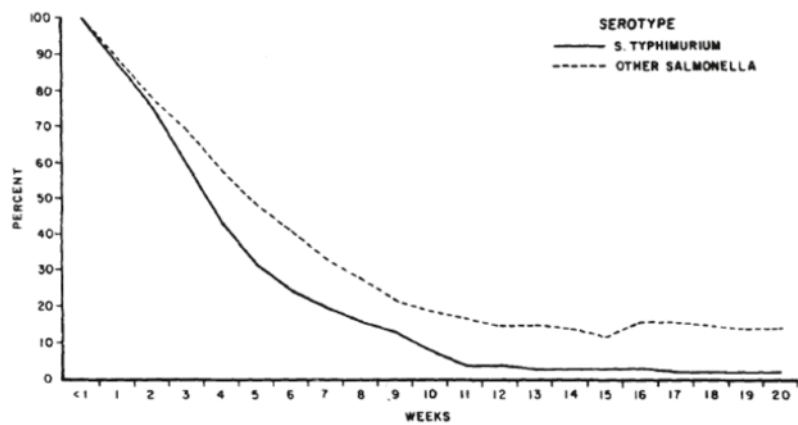
2.6 Treatment

Recommendations for the treatment of acute gastroenteritis due to NTS vary (Table 2.3). The rationale for treatment of both acute NTS gastroenteritis and carriage will be the focus of the remainder of this review. Recommendations for the treatment of extra-intestinal complications of NTS are available elsewhere [4].

Antibiotic agents with *in vitro* and *in vivo* activity against NTS include ampicillin/amoxicillin, trimethoprim-sulfamethoxazole, tetracyclines, third generation cephalosporins, macrolides and fluoroquinolones. Aminoglycosides show good *in vitro* activity but poor clinical efficacy and are not recommended [9, 45]. Ciprofloxacin and other fluoroquinolones have previously been restricted in children due an association with arthropathy, first described in young beagles

[46]. However, a systematic review of the safety of ciprofloxacin in children has found that musculoskeletal adverse effects are infrequent (risk 1.6 %, 95 % CI 0.9–2.6 %), generally mild and usually reversible [47].

2.6.1 NTS Gastroenteritis

A Cochrane review of trials investigating antibiotic treatment of NTS concluded that there was no evidence to support antibiotic therapy in otherwise healthy children and adults with non-severe diarrhoea [58]. It included 12 randomised controlled trials (RCT) published before 1998, of which only five reported on clinical outcomes that were extractable for meta-analysis. Importantly, almost all trials excluded infants less than 6 weeks and patients with underlying disease and

Table 2.3 Recommendations for the treatment of acute NTS gastroenteritis. (With duration of treatment and/or specified antibiotic choice when stated in source)

Source	Age <3 months	Severe infection	Immuno-compromised	Haemo-globinopathies	Chronic GI disease	Other
Infectious diseases and paediatric reference books						
Textbook of Pediatric Infectious Diseases (Feigin and Cherry) [48]	Yes, 5d	–	Yes, 5d	Yes, 5d	–	–
Forfar and Arneil's Text-book of Pediatrics [49]	Yes	–	Yes	–	–	–
Evidence-based Pediatric Infectious Diseases (Isaacs) [50]	Yes, 5–7d, cipro-floxacin or azithro-mycin (empiric) and amoxicillin if susceptible	Yes, 5–7d	Yes, 5–7d	Yes, 5–7d	Yes, 5–7d	Malnourished, 5–7d
Principles and Practice of Pediatric Infectious Diseases (Long) [51]	Yes	–	Yes	–	Yes	–
Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases [4]	Yes, 2–3d	–	Yes, 7–14d, fluoroqui-nolone	–	–	Valvular heart disease; joint disease, 2–3d
Nelson Textbook of Pediat-rics [52]	Yes, 7–14d	–	Yes, 7–14d	Yes, 7–14d	–	–
Oski's Principles and Prac-tice of Pediatrics [53]	Yes	–	Yes	Yes	–	Malnourished
Red Book: 2009 Report of the Committee on Infec-tious Diseases [12]	Yes, as per susceptibilities	–	Yes, as per suscepti-bilities	Yes, as per suscepti-bilities	Yes, as per suscepti-bilities	–
Infectious Diseases of the Fetus and Newborn (Remington & Klein) [21]	Yes, 3–5d	–	Yes, 3–5d	–	–	–
Rudolph's Pediatrics [54]	Yes	–	Yes	–	–	–
The Sanford Guide to Anti-microbial Therapy [45]	Yes (<1y), 7–10d, fluoroquinolone	Yes[b], 7–10d, fluoroqui-nolone	Yes, 14d, fluoroqui-nolone	Yes, 7–10d, fluoroqui-nolone	–	Haemodialysis; vascular aneu-rysm; prosthetic joints, 7–10d, fluoroquinolone
Other sources						
IDSA Guidelines 2001 [55]	Yes (<6m), 3–7d	Yes, 3–7d	Yes, mini-mum 14d	–	–	Valvular heart disease, 3–7d
NICE guidelines: Diar-rhoea and Vomiting Caused by Gastroenteritis [56]	Yes (<6m)	–	Yes	–	–	Malnourished
UpToDate [57]	Yes, 3–10d	Yes[a], 3–7d	Yes, mini-mum 1d	Yes, mini-mum 14d	–	–

[a] Defined as high fever, severe diarrhoea (>9 stools per day), need for hospitalisation
[b] Defined as hospitalised with fever and severe diarrhoea

severe illness. Five studies were in infants and children ($n = 258$), with only one study including infants less than 4 weeks [59–63]. The review did not identify significant differences in length of illness, diarrhoea or fever between any antibiotic regimen and placebo. Furthermore, although antibiotics were associated with more negative stool cultures during the first week of treatment, clinical relapse was more common and there were more cases of positive cultures at three weeks in the antibiotic-treated group. Adverse drug reactions, including rash, gastrointestinal upset and headache, were also more common in the antibiotic group [58]. Given the exclusion criteria, these results can not be extrapolated to patients at higher risk of severe and invasive infection.

Although the treatment of patients with risk factors for invasive disease seems reasonable, a benefit has not been documented in a RCT. This paucity of data likely explains the discrepancies in treatment recommendations between guidelines and reference books detailed in Table 2.3. Despite this variability, recommendations for the treatment of infants under 3–6 months of age and immunocompromised patients are consistent between all identified references. A 1988 consensus statement for the management of *Salmonella* infections in the first year of life similarly recommends treatment in infants less than 3 months following a blood culture irrespective of the severity of illness [64]. Other conditions where treatment has been recommended, albeit less consistently, include haemaglobinopathies, chronic gastrointestinal disease (for example inflammatory bowel disease), severe infection, malnourished state and vascular or joint disease.

Agents for treatment of NTS can be divided into non-absorbable (colistin and neomycin), absorbable (ampicillin, amoxicillin, tetracycline, macrolide and trimethoprim-sulfamethoxazole) and those with potent intra-cellular activity (fluoroquinolones) [58]. As *Salmonellae* are intracellular pathogens, it is postulated that the latter may be more effective in treatment of this infection and is specifically recommended for treatment of immunocompromised patients in two sources [4, 45]. The Cochrane review included eight trials involving fluoroquinolones [58]. Subgroup analysis showed no benefit of treatment with fluoroquinolones, although negative stool cultures in the first week of treatment were more likely with fluroquinolone treatment compared to other absorbable antibiotics. Consequently, as there are insufficient data to recommend a particular agent, empiric treatment should be based on local susceptibility data and modified according to susceptibility results.

The optimal duration of treatment of NTS gastroenteritis has not been studied. Recommendations vary amongst sources between 3 and 14 days depending on the underlying condition (Table 2.3). Of the trials included in the Cochrane review, duration varied between 1 and 14 days, with 5 days being the most common treatment regimen. The authors commented that the two studies with longer antibiotic treatment duration (10 and 14 days) showed an apparent benefit of antibiotics at 8–21 days [58]. Subsequent to this review, a study reported that 10 days of ofloxacin compared with 5 days resulted in earlier eradication of NTS without an increase in carriage [65]. However, this study was relatively small and did not include a placebo arm.

Other trials have also been published since the last Cochrane review update in 1999 with conflicting results [66, 67]. An updated meta-analysis is awaited [68].

2.6.2 Convalescent NTS Carriage

Treatment of convalescent NTS carriage in children has not been subject to RCT but is generally not recommended (Table 2.4).

2.6.3 Chronic NTS carriage

There are no RCT investigating the treatment of chronic NTS carriage in healthy children. In adults with chronic carriage, norfloxacin and azithromycin were no better than placebo in eradicating carriage and were associated with higher rates of re-infection and selection of drug-resistant isolates in endemic areas [69]. However, although antibiotics have been shown to prolong NTS excretion, this paradoxical finding may only

Table 2.4 Recommendations for treatment of convalescent and chronic NTS. (With duration of treatment and/or specified antibiotic choice when stated in source)

Source	Convalescent excretion	Chronic carriage (> 1 year)
Infectious diseases and paediatric reference books		
Textbook of Pediatric Infectious Diseases (Feigin and Cherry) [48]	–	Not recommended
Forfar and Arneil's Textbook of Pediatrics [49]	Not recommended	–
Evidence-based Pediatric Infectious Diseases (Isaacs) [50]	Not recommended	
Principles and Practice of Pediatric Infectious Diseases (Long) [51]	–	–
Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases [4]	'Not recommended for uncomplicated convalescent excretion'	'Treatment of persons with asymptomatic carriage of NTS is controversial'
Nelson Textbook of Pediatrics [52]	–	–
Oski's Principles and Practice of Pediatrics [53]	Not recommended	Not recommended
Red Book: 2009 Report of the Committee on Infectious Diseases [12]	–	Not recommended
Infectious Diseases of the Fetus and Newborn (Remington & Klein) [21]	Not recommended for infants	–
Rudolph's Pediatrics [54]	–	–
The Sanford Guide to Antimicrobial Therapy [45]	–	–
Other sources		
IDSA Guidelines 2001 [55]	–	–
NICE Guideline: Diarrhoea and Vomiting Caused by Gastroenteritis [56]	–	–
UpToDate [57]	–	Recommended for all patients. Duration: 4–6 weeks; fluoroquinolone
		HIV infection: 'consider suppressive prophylactic therapy if failure to eradicate'

be relevant following treatment of acute NTS infection. In the Cochrane review, only three trials included asymptomatic patients (7.2 % of all participants) and separate data were not available for subgroup analysis [58].

Treatment of chronic NTS carriage is recommended in one source, based on data for the eradication of chronic *Salmonella* Typi and Paratyphi [57, 70] (Table 2.4). In patients with HIV infection, prolonged ciprofloxacin (minimum 3 months) may also reduce NTS relapse [71] and up to 6 months suppressive treatment is recommended for HIV-infected adolescents and adults with recurrent NTS bacteraemia [72].

2.7 NTS and Pregnancy

Pregnancy does not appear to increase the risk of maternal NTS infection. Screening of a large cohort of pregnant women (30,471) at the time of delivery detected NTS in only 60 (0.2 %), of which 17 (28 %) were symptomatic [73]. Of the 60 babies born, only seven (11.7 %) excreted NTS and five (8.3 %) had gastroenteritis. Transplacental infection can occur in the setting of maternal NTS bacteraemia and is often lethal to the foetus [74]. Transmission of NTS through breast milk has also been described, including three nursery outbreaks due to infected pooled milk [75]. More

Table 2.5 Infection control recommendations

Source	Recommendation
Infectious diseases and paediatric reference books	
Textbook of Pediatric Infectious Diseases, (Feigin and Cherry) [53]	Hospitalised patient: standard and contact precautions until stool cultures are negative. Surveillance cultures on all neonates in nursery
Forfar and Arneil's Textbook of Pediatrics [49]	'No restriction of activities if stool is normal'
Evidence-based Pediatric Infectious Diseases (Isaacs) [50]	–
Principles and Practice of Pediatric Infectious Diseases (Long) [51]	Hospitalised patient: standard and contact precautions
Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases [4]	Hospitalised patient: standard and contact precautions
	Health care worker: exclusion until asymptomatic and passing formed stool
Nelson Textbook of Pediatrics [52]	–
Oski's Principles and Practice of Pediatrics [53]	–
Red Book: 2009 Report of the Committee on Infectious Diseases [12]	Hospitalised patient: Standard and contact precautions for diapered and incontinent children for duration of illness
	Child care: exclusion until diarrhoea resolves. Contacts do not require stool culture. Exclude symptomatic contacts until diarrhoea resolves
Infectious Diseases of the Fetus and New-born (Remington & Klein) [21]	Hospitalised neonate: standard and contact precautions. 'When two or more cases are recognized, environmental cultures, cultures of all infants, cohorting, early discharge of infected infants, and thorough cleaning of all possible fomites in the nursery and delivery rooms are recommended. If cases continue to occur, the nursery should be closed to further admissions'
Rudolph's Pediatrics [54]	–
The Sanford Guide to Antimicrobial Therapy [45]	–
Infection control sources	
CDC: 2007 Guideline for Isolation Precautions [95]	Hospitalised patient: standard and contact precautions for diapered or incontinent persons for duration of illness
NHMRC: Australian Guidelines for Prevention and Control of Infection in Healthcare [96]	Hospitalised patient: contact precautions for faecally incontinent patients for duration of illness
	Health care worker: exclusion until 24 h after symptoms have resolved
Other sources	
IDSA Guideline 2001 [55]	–
NICE guideline: Diarrhoea and Vomiting caused by Gastroenteritis [56]	Childcare/school: exclusion until 48 h after symptoms have resolved
UpToDate [57]	Healthcare workers: exclusion until diarrhoea resolves
	Food handlers: as above

recently, outbreaks associated with formula feed have been reported [76].

Treatment of asymptomatic maternal NTS carriage is unlikely to be of benefit, but should be considered for severe infection. There are no guidelines for empiric treatment of neonates with known maternal excretion. Due to the increased risk of severe infection in this group, consideration should be given to screening and treatment, as outlined previously, if NTS is detected in the stool [77].

2.8 Antibiotic Resistance

Antibiotic-resistant NTS are associated with increased treatment failure and risk of invasive disease [78]. Worldwide surveillance data has demonstrated an overall increase in antibiotic resistance among NTS, although significant geographical and serotype variability exist [79, 80]. The widespread use of antibiotics in food animals has been implicated in the increasing prevalence of antibiotic resistant NTS [81].

The European Centre for Disease Control (formally Enter-net) and the National Antimicrobial Resistance Monitoring System (NARMS) provide comprehensive surveillance data on rates of NTS drug resistance in Europe and the United States [82, 83]. Data from these studies, as well as reports from middle to low income countries, indicate high rates of ampicillin, amoxicillin and trimethoprim-sulfamethoxazole resistance, particularly among the globally prevalent serotypes *Salmonella* Enerididis and *Salmonella* Typhimurium [82–84]. Of particular concern is the emergence of extended spectrum beta-lactamase (ESBL) genes in NTS [80], as well as reports of carbapenemase-containing NTS isolates [85, 86], both of which confer high level antimicrobial resistance.

Fluoroquinolones and third generation cephalosporins are frequently used for the treatment of NTS that are resistant to conventional antibiotics, although reports of increasing resistance to these second line agents are emerging [80]. Isolates resistant to nalidixic acid are frequently resistant to fluoroquinolones, and this is a recommended screening test for ciprofloxacin resistance [3]. However, although a study found the nalidixic acid disk diffusion method was 100 % sensitive for the detection of reduced ciprofloxacin susceptibility (defined as MIC > 0.125 μg/ml) [87], there have been subsequent reports of isolates that are susceptible to nalidixic acid but exhibit reduced susceptibility to ciprofloxacin [88].

2.9 Infection Control

Outbreaks of NTS have been reported in neonatal units, paediatric wards and child care facilities [11, 89]. However, despite the frequency of asymptomatic NTS excretion following acute infection, the risk of transmission from asymptomatic health care workers to patients appears to be small when strict adherence to hand hygiene is observed [90]. Similarly, transmission from asymptomatic food handlers is rare, with one survey identifying only 2 % of food handlers as the source of 566 NTS outbreaks [91].

Methods to control the spread of *Salmonella* include appropriate food preparation, water sanitation and strict hand hygiene. Most resources recommend exclusion of children, health care workers and food handlers from childcare/school or work until 24–48 h after resolution of symptoms (Table 2.5). For symptomatic hospitalised patients, standard and contact precautions are recommended. There are also some non-randomised data that suggest the prophylactic use of antibiotics (trimethoprim-sulfamethoxazole or ciprofloxacin) in addition to strict barrier nursing, may control nosocomial *Salmonella* epidemics [92–94]. Confirmation of clearance of NTS is generally not recommended.

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Invasive Fungal Infections in Patients with Chronic Granulomatous Disease

3

Stefanie Henriet, Paul E. Verweij, Steven M. Holland and Adilia Warris

Abstract

Invasive fungal infections are a major threat for chronic granulomatous disease (CGD) patients. The present study provides a comprehensive overview of published invasive fungal infections in the CGD host through an extensive review of epidemiological, clinical, diagnostic and therapeutic data. In addition to the often mild clinical presentation, the currently used diagnostics for invasive aspergillosis have low sensitivity in CGD patients and cannot be easily translated to this non-neutropenic host. *Aspergillus fumigatus* and *A. nidulans* are the most commonly isolated species. *A. nidulans* infections are seldom reported in other immunocompromised patients, indicating a unique interaction between this fungus and the CGD host. The occurrence of mucormycosis is mainly noted in the setting of treatment of inflammatory complications with immunosuppressive drugs. *Candida* infections are infrequently seen and do not cause mucocutaneous disease but do show an age-dependent clinical presentation. The CGD patient is susceptible to a wide range of fungal pathogens, indicating the need to determine the causative fungus, often by invasive diagnostics, to guide optimal and rational treatment. This review summarizes current understanding of invasive fungal infections in patients with CGD and will serve as a starting point to guide optimal treatment strategies and to direct further research aimed at improving outcomes.

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Table 3.1 Epidemiology of invasive fungal infections in CGD: comparison of published registry data

Geographical region [Reference]	Number of patients	*Aspergillus* infections (%)	Organ involvement (%)						AF prophylaxis (%)	Death (*Aspergillus*) (%)
			Lung	Skin	Liver	Brain	Bone	Septicemia		
Europe [18]	409	26	61	5	3	7	16	2	53	28
USA [4]	368	33	78	5	2	4	12	0	NA	35
UK [1]	94	27	85	NA	0	NA	10	0	93	50
Italy [20]	60	34	53	0	0	18	24	0	NA	50
Japan [19]	23	45	NA	NA	NA	NA	NA	NA	48	67
Spain [172]	13	20	NA	NA	NA	NA	NA	NA	77	25
Sweden [3]	21	24	86	NA	NA	NA	NA	NA	NA	0

AF antifungal, *NA* not available

3.1 Introduction

Patients suffering from chronic granulomatous disease (CGD) are well known to be prone to invasive fungal infection, thereby providing a unique opportunity to examine host susceptibility to fungal invasion within the framework of a well defined immune defect. CGD is a rare inherited disorder of the NADPH oxidase in which phagocytes fail to generate the microbicidal reactive oxidant superoxide anion and its metabolites, hydrogen peroxide, hydroxyl anion, and hypohalous acid. Clinically, as a result of the defect in this key innate host defense pathway, CGD patients suffer from recurrent life-threatening bacterial and fungal infections and inflammatory sequelae. The molecular basis of CGD is well understood: CGD is a genetically heterogenous disease caused by mutations in any of the five structural components of NADPH oxidase, including the membrane-bound glycoprotein p91$^{phox\ (phagocyte\ oxidase)}$ and p22^{phox} and the cytoplasmatic components p40^{phox}, p47^{phox} and p67^{phox}. Birth prevalence ranges from 1/450,000 in Sweden, 1/250,000 in the United States and Japan, up to 1/120,000 in the United Kingdom and Ireland [1–4]. Although the majority of CGD patients are diagnosed in early infancy, fungal infection as presenting symptom in adult patients has been reported [5–9]. The suspicion of the presence of CGD as an underlying condition should arise whenever the diagnosis of invasive aspergillosis (IA) is made in the absence of another known risk factor.

Aspergillus is one of the most important encountered pathogens [10, 11], and the life-time incidence of IA in children with CGD before the advent of specific antifungal prophylaxis varied between 25 % and 40 % [11, 12]; although since the introduction of azole prophylaxis the incidence seems to decrease [13]. In comparison, the incidence of aspergillosis in children undergoing allogeneic haematopoietic stem cell transplantation (HSCT) or who were treated for hematological malignancy, who typically have a very limited period of susceptibility, lies between 2 % and 6.8 % [13–17].

In more detailed information extracted from the published data of CGD patient registries, the percentage of patients who had at least one infectious episode caused by *Aspergillus* spp. ranges from 26 % in Europe [18] up to 46 % in Japan [19]. More importantly, independent of the epidemiology of fungal infections in the published data, in the cases of pulmonary infection, brain abscesses and osteomyelitis, *Aspergillus* spp. have been the most commonly isolated organisms [1, 4, 18, 20] (Table 3.1). The most common *Aspergillus* spp. affecting CGD patients is *A. fumigatus* [10, 21, 22] followed by *A. nidulans* [23]. Much less attention has been paid to *A. nidulans* as an opportunistic pathogen in humans until recent years, when it was recognized especially in patients with CGD or with histories suggestive of CGD. White et al. [24] suggested in 1988 that CGD patients were at greater risk for *A. nidulans* than other immunocompromised patient populations. Scrutiny of the microbiology database of CGD patients at the National Insti-

tute of Health (NIH, Bethesda, MD) over a 10 year-period revealed 6 cases of *A. nidulans,* compared to 17 cases of *A. fumigatus* [23]. In cases of osteomyelitis, *A. nidulans* was isolated in up to 50 % of the cases [23, 25]. Although *A. nidulans* behaves more virulently in CGD patients than *A. fumigatus*, solid information on the relative frequency of IA due to *A. nidulans* in CGD patients is lacking. *Candida* is a less frequently encountered pathogen in CGD and is reported as being responsible for invasive fungal infection in 6 % of all isolated microorganisms among CGD patients registered in the European database, in 10 % in a Swedish CGD population, and up to 14 % in the Italian CGD study [3, 18, 20].

Invasive mucormycosis has not been recognized in patients with CGD as frequently as IA [26, 27]. A review of 929 pediatric and adult cases of mucormycosis revealed 81 % of the individuals having an impaired immune status, but none of these were associated with CGD [28]. In a systematic review of 157 cases of mucormycosis in children, 86 % were associated with risk factors as neutropenia, prematurity, diabetes mellitus and ketoacidosis; but no underlying primary immunodeficiency was noted [29]. However, when CGD patients are subjected to further immune compromising conditions affecting T cell function, such as methotrexate or steroids, typically in the setting of management of their inflammatory complications, mucormycosis has been encountered [30].

Globally, specific data regarding the epidemiology of non-*Aspergillus*, non-*Candida* IFD in CGD patients are scarce or described as "Fungi: not identified". Little detailed information is given on the identification of the causative fungus, and available data are almost exclusively found as individual case reports, making it difficult to assess any epidemiological burden of those fungi within the CGD population.

As a cause of death, fungal infections caused by *Aspergillus* spp. have been responsible for one-third to half of all deaths in CGD patients [2, 4, 20]. Mortality rates due to IFD caused by other fungi are unknown but, since case reports are more common with severe infections, may be overestimated from their true incidence.

Overall, as disease pathology and progression are the results of the complex interaction between the fungal pathogen and the host defense, exploring these host-fungus interfaces of the different fungal pathogens in relation to the host, gives us insight and detailed understanding of this challenging frontline, in order to optimize diagnostic and therapeutic tools.

We reviewed the literature of invasive fungal infections in CGD through topics such as epidemiological data, mycological characterization and identification, clinical manifestations, diagnostic features and in vitro susceptibility data in order to assess these findings within a framework of our current understanding of IFD in CGD.

3.2 Methods

3.2.1 Search Strategy

A PubMed database search of the English-, French-, and Dutch-language literature from 1970–2010 was performed using keywords "chronic granulomatous disease" and "fungal", "mycoses", "asper-gillosis", "aspergillus", "candidiosis", "candida", "zygomycosis", "dermatophytosis", "scedosporium", "trichosporon", "paecilomyces" and "endemic mycoses". In addition, published data concerning diagnostic features and susceptibility testing were reviewed. All the articles found by this means were systematically reviewed and the references cited in the articles were screened for additional information and cases, or for any duplicate reports of the same patient.

3.2.2 Criteria for Inclusion of Case Reports

3.2.2.1 Criteria for Diagnosis of Chronic Granulomatous Disease

The phagocytes of CGD patients lack the ability to generate superoxide and its metabolites due to a defect in the NADPH oxidase complex. As a diagnostic tool multiple tests have been validated in literature: the semi-quantitative nitrobluetetrazolium test (NBT) [31]; the cytochromec

test—a quantitative test based on the reduction of c ferricytochrome—and assays using probes of which the chemiluminescent or fluorescent properties are altered by their reaction with reactive oxidants. More recently, a fluorescence assay has been introduced, which uses the conversion of dihydrorhodamine 123 (DHR) to rhodamine 123, in the presence of hydrogen peroxide, being detected by flow cytometry [32, 33]. In the past, patients were diagnosed as having CGD by the occurrence of an appropriate clinicopathologic syndrome as described by Berendes et al. [34] and by the investigation of neutrophil killing defects. The vast majority of the included cases of CGD are confirmed by use of either the NBT test, chemoluminescence, cytochrome c reductase or DHR 123 test. If the patient died before proper diagnostic tests had been performed, inclusion was accepted when the autopsy and histology reports were suggestive of CGD in association with a positive genetic test of a sibling or a first-degree relative. A highly suggestive postmortem analysis in the absence of any genetic argument was not included in this review.

3.2.2.2 Documentation of Infection

As there are no definitions to categorize invasive fungal infection in patients with CGD, the principles of the EORTC/MSG criteria for invasive fungal infections [35], which apply to patients with cancer, were followed. Host factors, clinical symptoms and mycological evidence were used to categorize cases as probable or proven invasive fungal infection. Obviously, CGD was the host factor associated with an increased risk for fungal infection. Invasive fungal infection was considered proven in CGD patients with a positive culture from a specimen obtained by an invasive procedure showing histological evidence of growing fungi. Cases were considered probable if radiological features were considered to be consistent with fungal infection by the radiologist and mycological evidence was obtained, including a positive culture or detection of circulating galactomannan or 1,3-beta D glucan. Cases without mycological evidence were not included in this review. In case of a negative culture during the patient's lifetime, inclusion required the demonstration of infection at autopsy by culture of the causative agent from the affected organs.

3.2.2.3 Localisation of Infection

Documentation of the primary site of the infection was required. The definitions used were the following. (i) Localized infections were defined by isolation of the fungus from a clinically infected site without positive blood cultures or evidence of dissemination to distant organs; (ii) Disseminated infections were defined by the isolation of the fungal isolate from two or more noncontiguous sites showing clinical or histological evidence of infection, and/or positive blood cultures; (iii) Infections with contiguous spread were defined by the isolation of the fungal isolate from at least one contiguous site showing clinical or histological evidence of infection; (iv) Fungemia indicated the presence of the fungus in the blood.

3.3 Results

We identified 116 reported cases fulfilling the inclusion criteria of invasive fungal infections in CGD patients between 1970 and 2010. The demographic and clinical characteristics are summarized in Table 3.2. One hundred twenty-seven fungal species were isolated: 44 (35 %) *A. fumigatus*, 23 (18 %) *A. nidulans,* 5 (4 %) *A. niger*, 1 *A. flavus*, 11 (9 %) species belonging to the phylum *Zygomycota* (mainly *Rhizopus* spp.), 10 (8 %) *Candida* spp., 8 (6 %) *Trichosporon* spp., 6 (5 %) *Paecilomyces* spp., 5 (4 %) *Scedosporium* spp., 4 (3 %) *Penicillium* spp., 2 *Acremonium* spp., 2 *Alternaria* spp., 1 *Inonotus* spp., 1 *Exophiala*, 1 *Chrysosporium* spp., 1 *Fusarium* spp., 1 *Microascus* spp. and 1 *Hansela* spp. (Fig. 3.1). No reports of endemic mycoses in patients suffering from CGD were found following the above described search strategies.

Table 3.2 Characteristics of invasive fungal infections in 116 CGD patients

Variable	Cases, n (%)	Mortality, n (%)
Median age in years (range)	10 (0–69)	
Male/female/unknown	103/10/3	24/4/2
X-linked	65 (56)	16 (25)
AR	22 (19)	6 (27)
p47phox	10	2
p67phox	2	1
p22phox	2	0
AR, no details	8	3
Genetics unknown	29 (25)	8 (28)
Mono-fungal infections	108	26 (24)
Mixed-fungal infections	8	4 (50)
Antifungal prophylactic treatment	22	
INF-γ prophylactic treatment	28	
Lung involvement	83	25 (30)
Bone involvement	47	10 (21)
Infecting organism ($n=127$)		
A. fumigatus	44	13 (30)
A. nidulans	23	6 (27)
Other *Aspergillus* spp.	6	3 (50)
Zygomycetes	11	6 (55)
Paecilomyces spp.	6	0
Scedosporium spp.	5	0
Trichosporon spp.	8	2 (25)
Candida spp.	10	4 (40)
Miscellaneous group	14	3 (21)

3.4 Fungal Infections in CGD: by Clinical Manifestation

3.4.1 Pneumonia

Fungal involvement of the lung can result in two major clinical manifestations: the first is the result of direct infection of the pulmonary tissue or the infection of lung cavities, the second is the ability to trigger an immunological reaction when the fungus is inhaled. The CGD patient can suffer from both clinical identities. However, the clinical course and diagnostic signs and symptoms of invasive fungal pulmonary involvement differ remarkably from the classical clinical spectrum derived from cancer patients.

Seventy-two percent of the reported fungal infections in CGD had lung-involvement. Some species were more prone to cause pulmonary infection, e.g. Zygomycetes (91 %), *A. nidulans* (78 %), followed by *A. fumigatus* (72 %). The majority of the trichosporiosis patients suffered from lung infections as did half of the patients infected with *Paecilomyces* spp. Among the patients with candidiasis, only two out of ten mentioned pulmonary involvement, which can be explained by the route of infection being the result of disruption of skin or mucosal membrane (endogenous) instead of inhalation of spores (exogenous).

Symptoms include cough, usually non-productive, fever, chest discomfort and progressive dyspnea. However, some species have the propensity to be quite indolent, e.g. the clinical onset of *A. nidulans* infections was, compared to *A. fumigatus*, often very mild with nonspecific symptoms. *Trichosporon inkin* infections have also been described with an asymptomatic or mild presentation. However, the masking of the symptoms in those cases might have been at least partially explained by concomitant immunosuppressive drug use. Hemoptysis, a relatively common symptom of angioinvasive aspergillosis or mucormycosis in the immunocompromised host, has not been reported in CGD patients. In line with this, the typical lung lesions observed in neutropenic patients, i.e., the air crescent and halo signs, have never been reported in CGD cases. Nevertheless, *A. fumigatus* can hematogenously disseminate to liver, skin, brain. In contrast, *A. nidulans* tends to spread by contiguity.

A distinct pulmonary fungal infection syndrome in CGD is acute fulminant fungal pneumonia, "mulch pneumonitis", a medical emergency highly associated with and probably pathognomonic for CGD. High-level exposure to aerosolized fungi, such as that which occurs during spreading of mulch, lawn care, gardening, haying or digging in soil, leads to rather stereotypical signs and symptoms of pneumonia with hypoxia and diffuse infiltrates on chest radiographs. This condition can deteriorate rapidly

Fig. 3.1 Distribution (%)
of 127 fungal isolates out
of 116 cases of inva-
sive fungal disease in
CGD reported between
1970–2010

- Aspergillus fumigatus
- Aspergillus nidulans
- Candida
- Zygomycetes
- Aspergillus "other"
- Trichosporon
- Paecilomyces
- Scedosporium
- Penicillium species
- Alternaria species
- Acremonium species
- Inonotus (Phellinus) tropicalis
- Exophiala dermatitidis
- Chrysosporium zonatum
- Fusarium solani
- Microascus cinereus
- Hansenula polymorpha
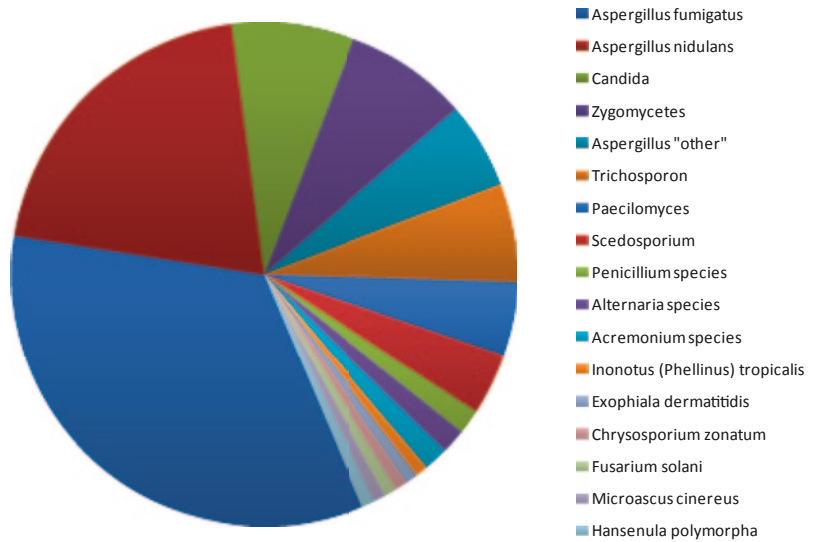
leading to acute hypoxemic respiratory failure, mechanical ventilation, and death. Since the first report in 1986 [36], 11 cases have been described [36–40]. Mean age was 23 years, ranging from 7–64 years, 4 out of 11 patients were previously well and mulch pneumonitis was the initial presentation leading to the diagnosis of CGD. In more than half of these cases multiple organisms were isolated, including *Aspergillus* spp. in association with members of the phylum Zygomyceta and/or *Penicillium* spp. *A. fumigatus* and *A. nidulans* were the only causative agents isolated in all cases of mulch pneumonitis. The mortality rate was high (8/11, 73 %) despite aggressive antifungal treatment, mechanical ventilation, extra-corporal membrane oxygenation and pulse corticosteroids (82 %) for the acute respiratory distress syndrome. However, it is obvious that with early recognition and prompt institution of antifungals to control the infection and steroids to regulate inflammation that response rates will improve. More recently developed immune regulatory agents might be of interest to further improve outcome. The pathological role of the agents of mucormycosis in this syndrome is unclear, and treatment directed at the *Aspergillus* component alone is probably adequate.

Aspergilloma and chronic necrotizing pulmonary aspergillosis have not been reported in CGD patients. Allergic bronchopulmonary asper-

gillosis (ABPA) in the CGD patient is rare, but has been reported [41]. However, it seems much more likely that what was described as ABPA in a CGD patient, acute respiratory failure after heavily mulching, should be more appropriately classified as "mulch pneumonitis".

3.4.2 Osteomyelitis

Bone-involvement was reported in almost half of the cases (41 %) of IFD in CGD, being the second most commonly encountered organ. *A. fumigatus* and *A. nidulans* were almost equally frequent (Table 3.3). However, in case of an *A. nidulans* IFD, osteomyelitis occurred in 74 % ($n = 17/23$), whereas *A. fumigatus* had a lower propensity to spread to the bone ($n = 18/44$, 41 %). It is very important to recognize that speciation of *Aspergillus* is an evolving field that has been transformed by molecular biological approaches, just as the other fields in microbiology has. The designation of *A. fumigatus* in most clinical laboratories is insentitive to subtle distinctions between *A. fumigatus sensu strictu* and organisms that have roughly similar growth and color characteristics but different clinical features, such as *A. viridinutans* [42].

As previously described by Sponseller et al. [43], fungal osteomyelitis is often the result of

Table 3.3 Fungal species distribution and localization of invasive infections in CGD patients

Organ involvement	Cases (%)	Species involved
Lung	83(72)	*A. fumigatus* (*n*=31)
		A. nidulans (*n*=25)
		Zygomycetes (*n*=9)
		Trichosporon spp. (*n*=7)
		Scedosporium spp., *Aspergillus* other (*n*=4)
		Paecilomyces spp. (*n*=3)
		Penicillium spp., *Candida* spp., *Acremonium* spp. (*n*=2)
		Exophilia dermatitis, Chrysosporium zonatum, Hansenula polymorpha (*n*=1)
Bone	47(41)	*A. nidulans* (*n*=17)
		A. fumigatus (*n*=18)
		Scedosporium spp. (*n*=3)
		A. niger, *Trichosporon* spp. (*n*=2)
		Paecilomyces spp., *Penicillium* spp., *Crysosporium zoantum, Inonotus tropicalis, Exophilia dermatitis* (*n*=1)
Brain	17(15)	*A. fumigatus* (*n*=9)
		A. nidulans (*n*=2)
		Zygomycosis (*n*=3)
		Scedosporium prolificans, Exophilia dermatitidis, Alternaria infectoria (*n*=1)
Skin	11(10)	*A. fumigatus, Paecilomyces* spp. (*n*=3)
		Fusarium solani, Microascus cinerius, Alternaria alternate, A. nidulans and *T. pullulans* (*n*=1)
Gastro-intestinal	11(10)	*Rhizopus* spp. (*n*=4)
		A. fumigatus, C. glabrata (*n*=3)
		Paecilomyces (*n*=1)
Lymphadenitis	11(10)	*A. fumigatus* (*n*=4)
		C. lusitaniae (*n*=2)
		A. flavus, A. nidulans, T. glabarata, C. albicans, H. polymorphae (*n*=1)
Meningitis	1	*C. lusitaniae* (*n*=1)

direct spread from a pulmonary focus towards adjacent chest-wall structures (64 %). Localized bone infection, without other organ involvement, was seen in only four subjects. Of these, *A. fumigatus* was isolated twice: a localized osteomyelitis of the midfoot [44] and an osteomyelitis of the patella and arthritis with positive culture of both tissue and the synovial fluid [45]. *A. nidulans*, later identified as *E. rugulosa*, was responsible for a femoral osteomyelitis [46] and the basiomycetous mold *Inonotus tropicalis* for a destructive sacral bone osteomyelitis with soft tissue abscesses [47]. None of these infections were preceded by trauma or apparent direct inoculation. The mortality rate was 21 %. Surgery was the cornerstone of treatment (72 %) and in combination with antifungal therapy the cure rate

was 62 %. However, more prospective research is needed to define optimal treatment strategies.

Few data are available about the clinical, histopathology and radiologic characteristics of fungal bone infections in CGD patients. In a brief report published by Galluzzo et al. [48], 14 CGD patients were compared with control subjects to determine osteomyelitis-related markers suggestive of CGD. Generally, multifocal osteomyelitis and/or simultaneous other organ involvement were the most relevant and statistically significant clinical findings suggesting CGD as an underlying disease. Histopathologic findings of chronic inflammation plus granulomata, multinucleated giant cells, histiocytes, or necrosis were significantly over-represented among CGD patients. In contrast, acute or chronic

inflammation plus granulation tissue, remodeled bone, or lymphocytes were significantly under-represented and infrequently found in CGD patients. The presence of extensive destruction in association with minimum sclerosis on the X-ray, almost invariably noted at the time of diagnosis, was also described previously by Wolfson et al. [49]. Whether the particular histopathologic and radiologic patterns described in osteomyelitis occurring in CGD patients are fungus dependent or CGD host dependent is unclear. Overall, the lack of early clinical signs of osteomyelitis in association with advanced destructive lesions—often multi-focal—and the isolation of a likely opportunistic pathogen, is highly suggestive for the presence of CGD as an underlying condition, especially if the osteomyelitis is associated with other organ involvement, or if there are his-topathologic signs of chronic inflammation and granulomata-related features.

3.4.3 Other Organ Involvement

The skin is less commonly infected by fungal than bacterial pathogens. The pathology is diverse, ranging from erythematous plaques, crusted, papulous, pustulous to purulent ulcers in the absence or presence of generalized symptoms as malaise and fever. The responsible pathogens are listed in Table 3.2. One case of linear IgA dermatosis has been described during IFD caused by *Paecilomyces* spp. [50].

Half of fungal skin lesions were preceded by a superficial abrasion of the skin or a minor trauma. The extremities were the most common locations. Primary cutaneous fungal infections can be the first manifestation of CGD. Mansoory et al. [9] described a chronic cutaneous *Fusarium* infection in a 54-year-old women with undiagnosed CGD. In such patients, milder CGD phenotypes likely due to residual levels of superoxide production are considered to be the reason for the unusually late manifestation [51]. Secondary cutaneous fungal infection, defined as the skin being an end-organ of hematogenous dissemination, is a much more aggressive clinical feature, resulting in massive dissemination and a very high case-fatality rate.

Fungal invasive gastrointestinal (GI) involvement in CGD is very rare, especially when compared to the extensive granulomatous involvement of the GI tract [52]. Etiologic agents are listed in Table 3.3. The clinical picture is that of hematogenous dissemination with abscess formation in liver, spleen and kidney, with exception of one isolated case of gastrointestinal mucormycosis that presented with an abdominal mass in a 10-month-old prematurely born infant [53]. In this boy, the diagnosis of gastrointestinal zygomycosis served as the presenting symptom of the underlying CGD.

Lymphadenitis, one of the classical presenting signs of CGD, has been rarely attributed to infection by fungi. Nevertheless, *Candida* lymphadenitis in the infant is very suspicious for CGD [54–56]. In case of *Aspergillus* infections, the lymph nodes involved are often those draining the site of infection.

Infection of the central nervous system was mainly observed as the consequence of a spread by contiguity from a primary pulmonary focus to the spinal cord. Localized brain abscesses are extremely rare but have been reported caused by *A. fumigatus*, *S. prolificans* and *Alternaria infectoria* [7, 57–59]. *Candida* spp. were responsible as causative agents in meningitis or ventriculitis [60, 61].

3.5 Fungal Infections in CGD: by Organism

3.5.1 *Aspergillus fumigatus*

Forty-four invasive *A. fumigatus* infections in CGD patients were reviewed. According to our criteria, 40 cases were proven IA and 4 were categorized as probable. Three out of the four patients with a probable IA presented with signs of symptoms of a mulch pneumonitis [37]. The majority of infections (37/44), were caused by a single fungus. In mixed fungal infections, the associated organisms were, *Rhizopus* spp., *A. niger*, *Penicil-*

lium spp., *Absidia corymbifera* and *C. dublinien-sis* [37, 38, 40, 62, 63]. Interestingly, in five out of the seven multiple-fungal infections a clear temporal relationship between massive exposure to molds, especially mulch, and the clinical presentation of pneumonia, was found [37, 38, 40]. The age, sex distribution and genetic profile did not differ from the overall demographic characteristics. Interferon-γ had been administered prophylactically to one third of the patients. However, prophylactic use of itraconazole was documented in only two cases. All of the invasive fungal infections caused by *A. fumigatus* were symptomatic, and accompanied by fever in 61 %. Detailed information on the individual clinical pictures is illustrated in Table 3.4.

Almost three-quarters had a history suggestive for primary lung invasion (30/44). Of these, half remained localized in the lungs [22, 36–38, 63–66] and more than one-third were disseminated, causing metastatic foci in bone, skin and/or the central nervous system [22, 67–74]. In 14 % of the *A. fumigatus* infections, the fungus had spread by contiguity from the lung to adjacent chest-wall structures [22, 70, 75–77].

The brain and the skin were the most common extra-pulmonary sites affected by *A. fumigatus*. Primary cutaneous aspergillosis due to *A. fumigatus* occurred in three cases, all of them in relation to direct inoculation of disrupted skin [78–80]. In four patients *A. fumigatus* caused isolated brain abscesses [7, 22, 57, 73]. It should be emphasized that the symptoms can be very indolent ranging from mild fever and headache in the absence of any localizing neurologic signs [57], up to isolated seizures mimicking intracranial tumor [58]. Isolated osteomyelitis without evidence for pulmonary involvement was reported twice [44, 45].

Susceptibility data were rarely provided. An elevated MIC for amphotericin B of 1.2 mg/l was found in an *A. fumigatus* isolate causing pneumonia and secondary osteomyelitis of the foot [69] and a MIC of 1.5 mg/l in a dual pulmonary infection with *A. fumigatus* and *C. dubliensis* [63]. A multi-azole resistant *A. fumigatus* isolate (MIC itraconazole > 16 mg/l, voriconazole > 16 mg/l, posaconazole 0.25 mg/l) causing osteomyelitis of the midfoot of a patient who received itracon-

azole prophylaxis, was treated successfully with extensive surgery and long-term oral posaconazole [44]. Patients with CGD may be at risk for azole-resistant fungi as they usually receive life-long azole antifungal prophylaxis [81–84].

Amphotericin B was the most commonly used first line treatment, followed by voriconazole. Half of the cases also underwent surgical interventions. If mentioned, adjuvant immuno-modulatory therapy, in the form of INF-γ and/or granulocyte transfusion was given in 27 %. The overall mortality rate was 30 %, mulch pneumonitis being responsible for more than half of the case-fatalities. However, as *A. fumigatus* has been the most commonly encountered fungal pathogen in CGD, it might be that less severe cases are not published, leading to overrepresentation of severe cases.

3.5.2   *Aspergillus nidulans*

Twenty-three cases of invasive fungal infections due to *A. nidulans* in CGD patients were previously reported and fulfilled the inclusion criteria of proven infection (*n* = 22) or probable infection (*n* = 1). In one patient, *A. nidulans*, *A. fumigatus* and *A. restrictus* were detected in sputum. However, no histological confirmation of multiple fungal infections was obtained [85].

The major clinical features of invasive *A. nidulans* infections in CGD patients are summarized in Table 3.5. Twenty-two patients were male, and the median age of the population was 8 years (range 3–21 years). Interestingly, of those patients whose genetic pattern was reported (*n* = 19), 90 % were X-linked gp91[phox], compared to 75 % in all documented cases and 74 % in those caused by *A. fumigatus*. One boy had a defect in p22[phox] and one girl had a defect in the cytoplasmic factor p67[phox]. Four patients had suffered from previous fungal infections by the time the invasive *A. nidulans* infection occurred.

The most common localization (74 %) of the reported *A. nidulans* infections in CGD patient was the lung, with direct spread to adjacent chest-wall structures (Fig. 3.2). In contrast to IFD caused by *A. fumigatus*, the presenting signs

Table 3.4 The major clinical features of *A. fumigatus* infections in CGD

Case [Reference]	Sex	Genetic type	Age (years)	Site of disease	Prophylaxis	Treatment	Surgery	Outcome
1 [22]	NM	AR	8	Lung, chest wall	TMP-SMX, INF-γ	AMB	Yes	Survived
2 [22]	NM	AR	6	Lung, liver, brain, ribs	TMP-SMX, INF-γ	AMB	No	Died
3 [22]	M	X-CGD	2	Brain	TMP-SMX, INF-γ	AMB, 5-FC	Yes	Survived
4 [22]	M	X-CGD	5	Hepatic abscess, chest wall, rib osteomyelitis	TMP-SMX, INF-γ	AMB, ITC	Yes	Died
5 [22]	NM	AR	4, 5	Lung	TMP-SMX, INF-γ	AMB, ITC	Yes	Died
6 [62]	M	NA	5	Chest-wall, thoracic vertebrae, spinal cord	NM	AMB	NA	Survived
7 [62]	M	NA	5	Lung, chest wall, thoracic vertebrae	No	AMB, 5-FC, MIC	No	Survived
8 [36]	M	X-CGD suspected FA	20	Mulch pneumonitis	No	AMB, 5-FC	No	Died
9 [67]	M	X-CGD suspected FA	10	Lung, lymphadenitis, femoral osteomyelitis	TMP-SMX	AMB, ITC	No	Survived
10 [68]	M	X-CGD	11	Lung, brain, bone (mastoid)	TMP, rifampicin	AMB, 5-FC, ITC, gran Tx	Yes	Survived
11 [126]	M	NA	18	Anterior chest wall, sternum osteomyelitis, mediastinum, extensive vascular invasement with mycotic pseudoaneurysm	NM	NM	Yes	Survived
12 [69]	M	X-CGD suspected FA	7	Lung, osteomyelitis foot, lymphadenitis draining Lnn	Ampicilline	AMB	Yes	Survived
13 [70]	M	X-CGD	18	Lung, ribs, humerus, chest wall	TMP-SMX	AMB, 5-FC, ITC	Yes	Survived
14 [71]	M	NA	15	Lung, humerus	No	AMB, 5-FC, ITC, gran Tx	Yes	Survived
15 [75]	M	X-CGD	10	Lung, rib, vertebrae, epidural soft tissue	TMP-SMX, INF-γ	AMB, ABLC, 5-FC, ITC, gran Tx	Yes	Survived
16 [64]	M	X-CGD	30	Lung	TMP-SMX, INF-γ	VOR	No	Survived
17 [65]	M	X-CGD	0	Lung	No	AMB	No	Survived
18[57]	M	X-CGD	11	Brain abscess	TMP-SMX	AMB, ITC	Yes	Survived
19 [44]	M	X-CGD	18	Osteomyelitis midfoot	TMP-SMX, INF-γ	VOR, CAS, POS	Yes	Survived
20 [76]	F	AR	14	Lung, pericard, myocard, endocard	No	AMBL, VOR, CAS, gran Tx	Yes	Died
21 [166]	M	X-CGD	3	Soft-tissue skull, parietal bone, brain parenchym	No	NM	Yes	NA
22 [80]	M	X-CGD suspected FA	3	Skin "sporotrichoid Aspergillosis", lung	No	AMB, VOR, ITC	Yes	Still not under control

Table 3.4 (continued)

Case [Reference]	Sex	Genetic type	Age (years)	Site of disease	Prophylaxis	Treatment	Surgery	Outcome
23 [79]	M	X-CGD	13	Skin	TMP-SMX, INF-γ	AMB, ITC	Yes	Survived
24 [72]	M	NA	7	Lung, skin	NM	AMB	NA	Died
25 [78]	M	X-CGD	19	Skin	TMP-SMX	ITC	No	Survived
26 [37, 38, 40]	M	p47phox	14	Mulch pneumonitis	No	VOR, gran Tx	No	Died
27 [38]	M	X-CGD	7	Mulch pneumonitis	No	AML, VOR, CAS	No	Died
28 [37]	F	p47phox	23	Mulch pneumonitis	No	VOR, CAS	No	Survived
29 [37]	M	X-CGD	20	Mulch pneumonitis	TMP-SMX	AMB	No	Died
30 [37]	M	X-CGD	23	Mulch pneumonitis	TMP-SMX, ITC	AMBL	No	Died
31 [37]	M	p47phox	64	Mulch pneumonitis	TMP-SMX, INF-γ ITC	AMB	No	Died
32 [37]	M	X-CGD	10	Mulch pneumonitis	TMP-SMX, INF-γ	AMB	No	Died
33 [66]	M	NA	3	Lung	NA	AMB	No	Survived
34 [66]	M	NA	8	Lung	NA	AMB	No	Survived
35 [66]	M	NA	9	Lung	NA	AMB	No	Survived
36 [39]	M	X-CGD suspected FA	32	Mulch pneumonitis	NA	gran Tx	No	Died
37 [167]	M	AR suspected, sister AR CGD	17	Vertebrae, ribs, cervical sinus, spinal cord	No	AMB, AMBL, ITC, CAS, ABLC, VOR	Drainage abscess, vertebral surgery not possible	Survived
38 [58]	M	p47phox	16	Brain abscess	No	AMBL, VOR	Yes	Survived
39 [7]	M	X-CGD	8	Brain abscesses, multiple	No	AMB, AMBL, VOR	No	Survived
40 [73]	M	X-CGD	20	Lung, osteomyelitis skull and subcutaneous abscess	TMP-SMX	AMB, gran Tx	Yes	Survived
41 [74]	M	X-CGD	4	Lung, osteomyelitis skull, epidural and intracerebral abscesses	No	AMB, 5-FC	Yes	Survived
42 [45]	M	NA	17	Arthritis, osteomyelitis patella	No	AMB, ITC	No	Survived
43 [63]	F	NA	6	Lung	No	AMB, VOR	No	Survived
44 [77]	M	X-CGD	4	Lung, rib, subphrenic abscess	NA	AMB, ITR	NA	Survived

AMB amphotericin B deoxycholate, *AMBL* amphotericin B liposomal, *ABLC* amphotericin B lipid complex, *ITC* itraconazole, *VOR* voriconazole, *POS* posaconazole, *KTC* keto-conazole, *MIC* miconazole, *CAS* caspofungin, *5-FC* 5-flucytosine, *NA* not available

Table 3.5 The major clinical features of *A. nidulans* infections in CGD

Case [Reference]	Sex	Genetic type	Age (years)	Site of disease	Prophylaxis	Treatment	Surgery	Outcome
1 [46]	M	NA	16	Osteomyelitis, long bone	INF-γ	ABLC, AMBL, ITC	Yes	Survived
2 [168]	M	NA	6	Lung, chest wall, vertebrae	TMP-SMX	AMB, ABLC, ITC	Yes	Died
3 [135]	M	X-CGD	20	Lung, 3th rib, femur, skull	NA	AMB, ITC, 5-FC	Yes	Survived
4 [43]	M	X-CGD	4	Lung, 8th–9th ribs, T6-L1 vertebrae	NA	AMB, AMBL, gran Tx	Yes	Died
5 [43]	M	X-CGD	9	Lung, 4th rib	NA	AMB	Yes	Survived
6 [43]	M	X-CGD	13	Progression of 4th rib lesion, T3–T4 with paraparesis	NA	AMB	Yes	Survived
7 [23]	M	X-CGD	6	Lung, pleura	No INF-γ	AMB, ITC, gran Tx	Yes	Survived
8 [23]	M	X-CGD	19	Lung, pleura, chest wall, vertebrae, skin, skull, brain	No	AMB, ABLC, ITC, 5-FC, gran Tx	Yes	Died
9 [23]	M	X-CGD	16	Lung, pleura, vertebrae, chest wall	KTC	AMB, ABLC, ITC, gran Tx	Yes	Survived
10 [23]	M	X-CGD	7	Lung, pleura, vertebrae, chest wall, sinuses, brain	NA	AMB, ABLC, gran Tx	Yes	Died
11 [23]	M	X-CGD	4	Lung	INF-γ until 1 mo before *A. nidulans* infection	AMB, ITC	Yes	Survived
12 [169]	M	X-CGD	8	Lung, rib	Clindamycin	AMB, gran Tx	No	Survived
13 [170]	M	NA	10	Lung, pleura, axillay abces, 2nd–3rd ribs, vertebrae	No	AMB	Yes	Survived
14 [86]	F	p67phox	3	Endocarditis, skin lesions, blood	TMP	AMB	Yes	Died
15 [128]	M	X-CGD	21	Lung, chest wall, spinal cord, T5–T7 vertebrae, popliteal abscess	TMP-SMX, ITC, INF-γ	AMB, AMBL, VOR, CAS, gran Tx	Yes	Died
16 [129]	M	X-CGD	4	Lung, T2–T5 vertebrae, spinal cord	TMP-SMX, ITC	AMB, AMBL, VOR, POS, CAS, gran Tx	Yes	Survived (*ex-vivo* gene therapy)
17 [85]	M	X-CGD	13	Lung	TMP-SMX, ITC but diarrhea and serum levels (−)	AMBL, VOR, CAS	No	Survived
18 [130]	M	X-CGD	5	Lung, chest wall cutaneous abscess	NA	AMB, VOR, ITC, gran Tx	Yes	Survived
19 [149]	M	X-CGD	8	Lung, 6th rib, chest wall fistula over the rib, psoas abcess	TMP-SMX, ITC stopped 4 wk prior to *A. nidulans*	AMB, AMBL, gran Tx	Yes	Survived (BM Tx)
20 [24]	M	X-CGD	4	Lung, chest wall, T8–T11 vertebrae, 7th rib	NA	AMB, 5-FC, ITC gran Tx	Yes	Survived
21 [171]	M	NA	5	Lung, chest wall, vertebrae, spinal cord syringomyelia	NA	VOR	Yes	Survived
22 [37]	M	X-CGD	16	lung, MULCH pneumonitis	TMP-SMX, INF-γ	AMB, ITC	No	Survived
23 [150]	M	X-CGD	18	lung, osteomyelitis rib, vertebra	TMP-SMX, ITC	AMBL, VOR, CAS	Yes	Survived (BM Tx)

AMB amphotericin B deoxycholate, *AMBL* amphotericin B liposomal, *ABLC* amphotericin B lipid complex, *ITC* itraconazole, *VOR* voriconazole, *POS* posaconazole, *KTC* ketoconazole, *CAS* caspofungin, *5-FC* 5-flucytosine, *NA* not available, *gran Tx* granulocyte transfusion, *BM Tx* bone marrow transplantation
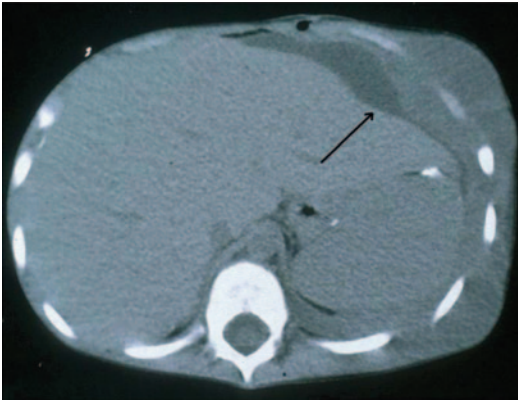
Fig. 3.2 Computed tomography scan of *A. nidulans* infection in a patient with chronic granulomatous disease. (The extensive chest wall invasion and subcutaneous infiltration (*arrow*))

and symptoms were often mild with low grade fever, local pain or swelling, malaise and cough, but could even be completely asymptomatic, with undetected lung infiltrates. Vertebrae, mostly thoracic, were the most frequent sites of bone involvement (47 % of cases), while the ribs and skull were involved in 39 % and 9 % of the cases, respectively. Invasion of the vertebrae was accompanied in 45 % by clinical signs and symptoms of spinal cord invasion. Two more exclusive cases were noted: Casson et al. [86] reported an *A. nidulans* endocarditis in a 3-year-old girl with CGD who also had an atrial septal defect. Extensive prior investigation by local biopsy and repeated fungal antibody titers were negative and diagnosis was made on a second culture in which the organism grew from a mass obtained in the right atrium. Subsequently, *A. nidulans* was grown from embolic skin lesions and the site of the sternotomy. Despite extensive surgery and antifungal treatment, blood cultures grew *A. nidulans* and she died 24 days post-surgery. In 2003, an unusual mild and localized course of the disease was described in a 16-year-old male with CGD presenting with femoral osteomyelitis, without pulmonary involvement [46]. No blunt or penetrating trauma was noted. Treatment with liposomal amphotericin B and granulocyte colony-stimulating factor as well as extensive surgical debridement, followed by prolonged treatment with itraconazole resulted

in an excellent clinical response. Microscopic characteristics include very short conidiophores and the presence of ascospores with two equatorial crests. However, after molecular typing in 2008, this showed to be caused by *A. rugulosa*, originally identified as *A. nidulans* based on microscopic features [87].

A. nidulans has a unique predilection for CGD. It remains a rare pathogen among all other immunosuppressed patients at high risk for IA [88–90].

3.5.3 Other *Aspergillus* Species

A. fumigatus is the primary agent of invasive human fungal infections, followed by *A. flavus*, *A. terreus*, and *A. niger* [12, 91–93]. In contrast, among CGD patients, *A. fumigatus* and *A. nidulans* are by far the most commonly isolated *Aspergillus* spp., followed by *A. niger* (*n* = 5) and *A. flavus* (*n* = 1) [22, 37, 62, 94].

Most of the non-fumigatus non-nidulans *Aspergillus* spp. were isolated in mixed fungal infections, and associated to *A. fumigatus* or *Rhizopus* spp. [37, 62]. The clinical picture related to multiple fungal species was that of mulch pneumonitis in half of the cases.

Kaltenis et al. [94] described a 10-year old child suffering from disseminated aspergillosis associated with systemic amyloidosis. The causative agent was *A. niger*. Despite aggressive treatment with amphotericin B and surgical drainage, intravenous leukocyte transfusions, INF-γ and immunoglobulin infusions, the patient died of multiple organ failure. *A. flavus* was the only causative agent in a 7-year old boy suffering from pulmonary aspergillosis associated with lymphadenitis; infection was controlled by surgical resection, amphotericin B and itraconazole [22]. In vitro susceptibility data concerning these *Aspergillus* spp. were not reported.

Attention has to be drawn to the occurrence of *fumigati*-mimetic molds in unusual, chronic or refractory IA. *Neosartorya udagawae* and *A. viridinutans* have been reported in CGD patients. Phenotypical they resemble *A. fumigatus*, but the course of the disease is distinctive from typical

aspergillosis, being chronic and spreading in a contiguous manner across anatomical planes, and relative refractoriness to antimycotic drugs. Although encountered in the CGD patient, *fumigati*-mimetic molds are not confined to CGD; unlike *A. nidulans* that is essentially restricted to this host. Correct genotypic-based identification of *fumigati*-mimetic fungi may have implications for clinical course and management [42, 95].

3.5.4   Non-*Aspergillus* Species

3.5.4.1   Zygomycetes
Mucormycosis refers to a group of opportunistic invasive fungal infections caused by fungi of the order Mucorales, predominantly of the genus *Rhizopus*, *Mucor* and *Absidia*.

Eleven cases of mucormycosis in CGD patients have been reported. Among them, nine *Rhizopus* isolates were identified [30, 37, 53], one *A. corymbifera* [40], and in one report, published in 1976, the clinical manifestation was described as "Phycomycosis", indicating the causative species as belonging to the phylum *Zygomycota* [96].

Two major clinical identities were noted: "mulch pneumonitis" (*n*=5) [37, 38, 40] and "invasive pulmonary mucormycosis" (*n*=5) [30, 96]. Dissemination occurred in three patients. *Mucorales* are the second most commonly occurring species found in mulch pneumonitis, mostly in association with *Aspergillus* spp. The association of mucorales and mulch pneumonitis in CGD seems to be related to the fact that both mucorales and aspergilli are found in the same demographic or environmental niche.

Invasive pulmonary mucormycosis can progress to thoracic wall abscess or nodular infiltrates of lung, spleen and brain. Attention has to be drawn to the use of prolonged steroid-based intense immunosuppressive regimes prior to the onset of mucormycosis. Median age was high, 18 years, which might well be explained by the fact that the inflammatory sequelae needing anti-inflammatory therapy develop with age in CGD. The prescription and prolonged use of steroid-based intense immunosuppressive regimes seems to predispose those CGD patients to "less typical" fungal infections, such as mucormycosis.

A particular case of localized gastrointestinal mucormycosis in an eight-month-old infant was reported by Dekkers et al. [53]. The patient presented with an abdominal tumor associated with fever and diarrhea and *Rhizopus microsporus var. rhizopodiformis* was cultured within 24 h from tissue specimens taken during laparotomy. Analysis of his neutrophil function confirmed CGD. The use of antacids might have contributed to the development of invasive mucormycosis by increasing the pH in the stomach [53]. Voriconazole and itraconazole are not effective treatment options for mucormycosis. The first line treatment was amphotericin B in most of the cases. However, in two patients with mulch pneumonitis, voriconazole was used together with caspofungin, without coverage for zygomycetes. Overall mortality was high (54 %) compared to mortality associated with IA in CGD, likely due to delays in diagnosis and delayed and inadequate use of steroids to down-modulate the acute inflammatory aspects of mulch pneumonitis.

3.5.4.2   *Candida* Species
The clinical spectrum of *Candida* infections in the immunocompromised host ranges from mucocutaneous disease to systemic life-threatening infections. Mucocutaneous involvement is remarkably rare in CGD. One report mentions an achalasia-like esophageal motility disorder as a result of a previously experienced *Candida* esophagitis [97], although this has not been observed in other diseases. Overall, in the CGD patient, *Candida* was the most common cause of meningitis, fungemia and lymphadenitis [54–56, 61, 98], but is not a causative agent of pneumonia, keratitis, hepatic abscesses and disseminated disease [10, 60, 63, 99, 100].

Young children appear more prone to develop *Candida* lymphangitis, blood stream infections and meningitis than older patients. Among the proven cases of reported meningitis, candidemia and lymphadenitis with or without dissemination, the age ranged from 8 weeks to 4 months. Clinical signs were those of a septic infant, febrile, irritable, sometimes associated with

local signs of organ involvement like lymphadenopathy, hepatosplenomegaly or signs of CNS involvement. The isolation of *Candida* spp. in an "otherwise healthy infant" was often the clue to diagnosis of a primary immunodeficiency defect. A possible explanation might be the immature mucosal immunity of the infant in the absence of a well-established tolerance for the *Candida* pathogen superposed to a defect in the phagocytic defense mechanisms.

The second risk group includes older children and adults in whom the "classic" risk factors, such as prolonged broad spectrum antimicrobial drug use (3/5 for having bacterial co-infection), use of intravascular catheters and prolonged intensive care stay or hospitalization, may be present.

Detailed information on *Candida* spp. distribution in CGD patients is not available. According to the individual reports, *C. glabrata* and *C. lusitaniae* were each isolated three times [10, 54, 55, 61, 98, 100], *C. albicans* was suspected twice according to microscopic features or positivity of antigen testing and the occurrence of antibodies [56, 99]; in one case no species identification was given [60]. Susceptibility testing was rarely performed. Species identification is important with respect to the intrinsic resistance profiles of various *Candida* spp., most notably the non-*albicans Candida* spp. Amphotericin B resistance has been reported in cases of *C. lusitaniae* infections. Four of the 11 CGD patients with invasive *Candida* infections died, three of them in the neonatal period due to a severely disseminated disease.

3.5.4.3   *Paecilomyces* Species

In CGD patients, *Paecilomyces* spp. have been reported as the third most common cause of osteomyelitis after *Serratia* spp. and *Aspergillus* spp. [4]. Cultured-proven infections caused by *Paecilomyces* spp. in CGD have been nicely reviewed by Wang et al. [8] in 2005. One additional probable pulmonary infection was described in a 12 year old boy receiving cyclosporine treatment for severe colitis and peri-rectal disease [101]. In total six cases have been reported of which four showed primary extrapulmonary disease. One of the extra-pulmonary cases was related to traumatic inoculation through a minor trauma. All of

them were symptomatic and the clinical picture did not differ from those reported in the non-CGD host.

P. variotii has been shown to be the causative agent in four cases: two cases of soft-tissue infection of which one was associated with multifocal osteomyelitis [102, 103], one case developed isolated splenic abscess [8] and one pneumonia [101]. *P. lilacinus* was reported only once in a soft-tissue infection of the abdominal wall [104]. Sillevis Smitt [50] implicated a *Paecilomyces* spp. in a pulmonary infection of a CGD patient also suffering from bullous Ig-A dermatosis.

Differentiation between the two species is extremely important, since *P. lilacinus* and *P. variotii* seem to present marked differences in their in vitro susceptibilities. Amphotericin B, itraconazole, and echinocandins showed poor activity against *P. lilacinus*, while the new triazoles, voriconazole and posaconazole show in vitro activity. In contrast, *P. variotii* was susceptible to most antifungal agents apart from voriconazole and ravuconazole [105]. All of the patients recovered; however, two were still under treatment by the time their report was published and one patient died after 4 months due to an *Aspergillus* pneumonia.

De Ravin et al. [106] raise the possibility that some of the *Paecilomyces* infection in CGD patients in previously published reports may have been caused by the emerging pathogen *Geosmithia argillacea*. When *P. variotii* is identified phenotypically, molecular approaches should be used to exclude *G. argillacea*, as infections observed in CGD are often aggressive, refractory to medical treatment and associated with a high fatality rate.

3.5.4.4   *Scedosporium* Species

S. apiospermum (previously known as *Pseudoallescheria boydii*) and *S. prolificans* represent important multidrug-resistant opportunistic pathogens. Both pathogens have been described in CGD patients as breakthrough infections during treatment with amphotericin B and voriconazole, or after long-term treatment for *Aspergillus* infections [107, 108].

In these patients, *S. apiospermum* caused pulmonary infections with both contiguous spread to adjacent bone and central nervous system, and/or hematogenous dissemination. Outcome was favorable after extensive surgery of the infected tissues and antifungal therapy including azole derivatives, itraconazole and voriconazole, and INF-γ. *S. apiospermum* is generally considered resistant to amphotericin B, while extended-spectrum triazoles are active *in vitro* and voriconazole as well as posaconazole have been used successfully for the treatment of CNS abscesses [109–111].

Bhat et al. [107] described the development of a *S. prolificans* brain abscess in a 23-year-old CGD patient with a history of recurrent pulmonary aspergillosis who was on maintenance voriconazole and interferon-γ. MRI revealed a right frontal lobe lesion and CT-guided biopsy yielded non-specific yeast forms without hyphal elements. Cultures identified *S. prolificans*. This pathogen is considered resistant to virtually all antifungal agents, including the extended-spectrum triazoles and the echinocandins [109]. The isolate showed high MICs for amphotericin B (>16 mg/l) and 5-FC (>16 mg/l). Although terbinafine does not appear to be active alone [112], the combination of voriconazole and terbinafine appeared synergistic and was used to treat the case, resulting in complete resolution of the brain abscess [107].

3.5.4.5 *Trichosporon* Species

The eight cases of *Trichosporon* infection described in patients suffering from CGD include three due to *T. pullulans*, although one isolate was subsequently identified as *Cryptococcus albinus*, a likely contaminant [113, 114]. *T. pullulans* has been reported as being a human pathogen, but controversy exists regarding whether *T. pullulans* causes invasive infection in patients with CGD [114]. It is unlikely that *T. pullulans* causes invasive disease in patients with CGD, nor is it a significant invasive infectious agent in other immune compromised humans.

The case described by Lestini et al. [115], highlighted how difficult it may be to treat CGD colitis with a high dose of immunosuppressive drugs, including prednisone and infliximab:

opportunistic organisms with high mortality and morbidity soon occur in such patients.

The four remaining *Trichosporon* spp. were identified as *T. inkin*, based on microscopic characteristics and inoculation on commercially available yeast identification systems. Clinically, *T. inkin* infection presented with pulmonary infiltrates. In three cases, the individuals were asymptomatic; the pulmonary infiltrates were found on routine chest radiographs. In one case, the lung lesions spread directly to adjacent ribs and penetrated the chest wall. Variable susceptibility to amphotericin B has described, and in general this agent lacks fungicidal activity against *Trichosporon*. The outcomes with voriconazole have been curative (S. M. Holland, unpublished).

3.5.4.6 Miscellaneous

A few other species of fungi have also been reported to cause invasive infections in CGD patients. Among these species, *Penicillium* spp. have been reported most commonly (*n*=4) [37, 116, 117]. However, it is now clear that many if not most of the isolates identified as invasive *Penicillium* in CGD are in fact due to the morphologically similar but treatment resistant *Geosmithia argillacea* [106]. *Acremonium* spp. (*n*=2) [118, 119] and *Alternaria* spp. (*n*=2) [59, 120] cause occasional infections as well. *Inonotus, Chrysosporium, Exophiala, Fusarium, Microascus* and *Hansenula* spp. have been reported once [9, 47, 121–125]. All of these cases fulfilled the criteria of a proven IFD, with the exception of one mulch pneumonitis, in which a bronchoscopic specimen grew *A. fumigatus*, *A. niger* and *Penicillium* spp. [37].

The majority of patients presented with localized infections, two of them with spread to adjacent structures. The lung was the most commonly involved organ, skin and soft-tissue infections were observed in three cases, osteomyelitis and cerebral abscess were each reported once. With exception of a 4-month-old infant who suffered from a localized pneumonia caused by *Acremonium kiliense* [119], the age at onset was quite high. Half of the patients were adults (median 18, range 0–69). Seven patients had X-linked CGD, four were diagnosed with autosomal recessive CGD and in three patients the genetic mutation

was not reported. Five patients each had received itraconazole prophylaxis and interferon-gamma prophylaxis. Amphotericin B was used in 12/14 cases as first line treatment, 2 of them also received miconazole or voriconazole combination therapy. One patient received itraconazole. The mortality rate was 21 %. Two patients died of multiple organ failure related to mulch pneumonitis and one patient succumbed to cerebral alternariosis [59].

3.6 Diagnosis

3.6.1 Culture and Biopsy of Clinical Specimens

One hundred and eight of the 116 patients (93 %) reviewed in this study fulfilled our criteria for proven IFD and 8 patients for probable IFD. The proportion of proven cases was much higher than observed in other risk groups, such as patients with hematological malignancy. The tendency to prefer proven cases for publication might cause a bias leading to a high proportion of proven cases. Invasive procedures may be higher in CGD patients compared to other patient groups because they are relatively in a better clinical condition and rarely have thrombocytopenia.

Recovery of fungi by culture of sterile material in association with consistent radiologic or clinical infectious disease process made the diagnosis in 101/108 cases. Blood cultures were positive in one mycotic pseudoaneurysm due to *A. fumigatus* [126], one *A. nidulans* endocarditis [86], fungemia due to *C. lusitaniae* [54] and *T. pullulans* [115]. Histopathologic and direct microscopic examination of a biopsy taken during open surgery was conclusive in the 7 additional proven cases [39, 59, 60, 85, 96, 108, 113]. Percutaneous fine needle biopsy was performed in 16 % (*n*=19), including 11 fine needle lung biopsies. Of these lung biopsies, all were conclusive, either on microscopic examination or culture. Molecular studies were helpful in diagnosing *Inonotus tropicalis* osteomyelitis of the sacrum using ultrasound guided bone biopsy [47].

In an era of multiple classes of antifungal agents, it is crucial to identify causative fungi to tailor therapy appropriately. Since 1970 numerous changes in the taxonomy of fungi have been implemented, especially through the use of molecular sequence information. Multilocus sequence-based phylogenetic analyses have emerged as the primary tool for inferring phylogenetic species boundaries and relationships within subgenera and sections. Recently, an international group of experts gathered for a workshop entitled "Aspergillus Systematics in the Genomic Era" and reviewed research data presented from research groups worldwide on recent genomic investigations, secondary metabolite analyses, multi locus phylogenetic analyses of the genus *Aspergillus*, and sequence based identification schemes for previously recognized human pathogens within the genus [127].

Sequence analyses of the ITS region appear to be appropriate for identification of *Aspergillus* isolates to the subgenus/section level [127]. Use of the ITS sequence will not provide sufficient sensitivity to discriminate among individual species within the section. At present partial β-tubulin or calmodulin are the most promising loci for *Aspergillus* identification to the species level. For the section *Nidulanti* the identification of clinical isolates by use of β-tubulin sequencing revealed that clinical isolates were commonly misidentified when solely relied on morphologic characteristics [87]. Differences in drug activity against the different species of the section *Nidulanti* was shown, underscoring the need for identification to the species level [87]. In addition to *Aspergillus* spp. the taxonomy of many other genera involved in fungal diseases in CGD has changed including *Candida*, *Paecilomyces*, *Scedosporium,* and *Trichosporon*. These changes will have impact on the epidemiology of fungal diseases in CGD patients, and the use of molecular techniques for fungal strain identification will increase our insight into specific pathogens and their susceptibilities in the near future.

3.6.2 Nucleic Acid Amplification, Antigens and Serology

In seven of the reported CGD cases, information about circulating *Aspergillus* antigen galactoman-

nan could be extracted [46, 65, 77, 99, 128–130]. In five of seven patients (71 %) circulating galactomannan could not be detected despite extensive invasive infection [46, 65, 77, 128, 129]. Interestingly, Verweij et al. [77] reported a CGD patient who developed invasive pulmonary aspergillosis and a subphrenic abscess. Fine needle aspiration of the subphrenic abscess recovered *A. fumigatus* as the etiologic agent. During treatment, high levels of *Aspergillus* antigen were detected in the abscess, but circulating antigen and *Aspergillus* DNA were undetectable in the serum.

van't Hek et al. [130] described circulating galactomannan in a 5-year-old boy with X-linked CGD suffering from invasive *A. nidulans* infection and chest-wall invasion in whom treatment with amphotericin B failed. The galactomannan ratio in serum remained high during amphotericin B treatment but started to decline 6 weeks after therapy with voriconazole was initiated and finally became negative after 4 months of treatment.

There has been a progressive increase in the understanding of the diagnostic utility of galactomannan which has enabled its incorporation into the EORTC/MSG consensus definitions and the ECIL guidelines [35]. Excellent performance characteristics in patients with hematological malignancies have been reported, with sensitivity up to 92.6 % and specificity of 95.4 % when serial monitoring of galactomannan was performed [131, 132], but the kinetics and predictive values of the antigen as a diagnostic marker in non-neutropenic hosts have not yet been defined [133]. The performance of galactomannan detection is the highest in the neutropenic host, while in non-neutropenic patients circulating galactomannan is detected in only a quarter of patients [134]. Possibly the rapid angio-invasive growth of *Aspergillus* in neutropenic patients facilitates the release of galactomannan into the blood. The poor performance of galactomannan detection in patients with CGD may be due to the fact that they are not neutropenic and the well-formed abscesses may preclude the release of the antigen into the blood [46, 77]. Furthermore, since galactomannan is an immunogenic molecule, it could be rapidly removed from the circulation in the non-neutropenic host.

In our review of IFD in CGD, information on diagnostic PCR was retrieved in six proven cases [46, 47, 59, 77, 85, 129]. Nucleic acid amplification of plasma and cerebrospinal fluid samples were all negative. In contrast, PCR performed on material obtained by biopsy of a brain lesion [59] and in the case of an osteomyelitis [47] revealed the etiological fungus.

Six cases mentioned strongly positive anti-*Aspergillus* antibodies [24, 64, 69, 77, 128, 135], even while circulating galactomannan was undetectable in repeated serum samples [128]. Except for the latter, no information was given about the assays used or about the epitopes recognized by these antibodies or corresponding antigen testing. In addition, antibody detection against *Scedosporium* spp. was helpful in two diagnoses [108], and once in a *C. albicans* sepsis [99]. Antibody responses to *Aspergillus* spp. may result from environmental exposure in the absence of disease, occur in the course of either aspergilloma or invasive disease, or be detected as part of a hypersensitivity syndrome. Secreted, cell wall and cytosolic fractions of *A. fumigatus* hyphae have been reported as potent B cell antigens [136]. Furthermore, work using the recombinant antigen mitogillin (*Aspergillus* protein toxin) suggests that specific antibody may be more prevalent in severely immunocompromised patients than has been described previously [137]. However at the moment, antibody detection is not considered useful for the diagnosis of IFD. Combined detection of *Candida* mannanaemia and anti-mannan antibodies may substantially contribute to the early diagnosis of candidosis in high-risk patients [138]. Nevertheless, little is known about the sensitivity of diagnostic modalities for IFD within specific immunocompromised patient groups as CGD. More work is required to define which diagnostic methods—or combination of methods—confer optimal predictive values in diagnosing IFD at an early stage in the CGD patient.

3.6.3 Radiologic Findings

Although pulmonary involvement was present in 72 % of the reported cases, beyond the description of "pulmonary consolidation" and "lung infiltrates", no specific signs as nodules, air crescent formation, halo signs, necrosis or cavitations were described or could be detected in the published images. Computed tomography and magnetic resonance scanning established extra-pulmonary extension to soft tissues, spinal cord injury and the extension of the osteolytic lesions. Local extension of disease from lung parenchyma to adjacent structures and osteomyelitis of the thoracic cage have been found particularly associated with underlying CGD. Unfortunately, not a single feature was pathogen-specific [139]. The absence of the characteristic diagnostic radiologic findings of IA in pediatric patients has been previously described by Burgos [93] in a multicentre retrospective analysis of 139 children with malignancy with or without hematopoietic stem cell transplantation. Thomas [139] performed a retrospective review of imaging performed in 27 consecutive patients with documented invasive aspergillosis, 12 of whom had primary immunodeficiency syndromes (including 6 CGD patients). Different pathophysiology in children compared to adults or, the exuberant granuloma formation in CGD in response to infection might explain the observed radiographic differences [64].

Pulmonary involvement is a common clinical feature of invasive fungal infections. This highlights the importance of identifying early, host-specific radiologic features on CT scan early in the course of disease. In the case of new or rapidly changing lesions, it is usually necessary and highly desirable to move to invasive diagnostic methods to identify the causative organisms.

3.7 Treatment

Overall, the combination of antifungal treatment and extensive and early surgical debridement was used in most patients. Almost all of the patients received presumptive initial treatment with amphotericin B (86 %). However, it is important to keep in mind that this series is accumulated from the era well before the advent of azole antifungals, and therefore is heavily weighted to amphotericin as the sole antifungal agent available. In general, the treatment guidelines for patients with aspergillosis and candidiosis can be followed in patients with CGD [140, 141] keeping in mind that non-fumigatus *Aspergillus* species, in particular *A. nidulans*, may require a different antifungal treatment. Furthermore, the use of adjunctive immunomodulatory treatment options as INF-γ and granulocyte transfusions, while ceonceptually robust in CGD is unproven and remains quite anecdotal.

Amphotericin B is active against the majority of clinically relevant yeasts and moulds isolated in the CGD patient, including *Candida* spp., *Aspergillus* spp. and Zygomycetes. However, the susceptibility of *Trichosporon* spp. to amphotericin B is variable and *Scedosporium* spp. are generally considered resistant [109].

Acquired and intrinsic resistance of *Aspergillus* spp. has been documented. Acquired azole-resistance is an emerging problem in *A. fumigatus* and may develop during azole therapy. This observation is of particular interest for CGD patients, as they should receive life-long itraconazole prophylaxis [82–84]. Development of resistance in *A. fumigatus* during azole therapy has recently been described in a patient suffering from CGD [81]. Non-*fumigatus Aspergillus* species, especially *A. terreus* and *A. nidulans*, may be intrinsically resistant to specific classes of antifungal agents [142]. Of particular interest is the resistance of *A. nidulans* to amphotericin B which might contribute to the high rate of treatment failure of these IFD in the era before azole antifungals [143]. In a clinical CGD study, the total dose of amphotericin B used in patients with *A. nidulans* infections was not only higher than for those with *A. fumigatus* infections (231 mg versus 56 mg, respectively), but also the duration of amphotericin B therapy was substantially longer, 220 days for *A. nidulans* infections compared to 65 days for *A. fumigatus* infections [23].

Voriconazole has recently been introduced as drug of choice of invasive aspergillosis in children and has been shown as successful salvage therapy in patients with CGD [144]. Among the described cases, use of voriconazole was first reported in 1998 and prescribed in 23 % of the cases. In comparison, 86 % of the cases received amphotericin B.

In an open-label trial of oral posaconazole, Segal et al. evaluated the effect in eight CGD patients suffering from refractory invasive mold infection including *P. variotii*, *S. apiospermum* and *A. fumigatus* infections. Posaconazole led to complete response in seven of the eight infections. In addition, Notheis et al. [129] described the beneficial effect in a deteriorating *A. nidulans* infection refractory to voriconazole and caspofungin treatment. Posaconazole shows promising results as safe and effective salvage therapy in CGD patients [145, 146].

Granulocyte transfusions were prescribed in 22 % of our cases, and/or 28 % received INF-γ. The use of granulocyte transfusions in CGD is supported by the observation that in vitro a small number of normal phagocytes may complement the oxidative defect and restore the killing ability towards *A. fumigatus* hyphae [147]. However, the efficacy of granulocyte transfusions is poorly documented and limited to case reports. The use of INF-γ as adjunctive therapy of IFD in CGD patients has not been investigated by controlled studies and remains controversial [148]. Three uncontrolled *A. nidulans* invasive fungal infections were cured either by ex-vivo gene therapy [129] or by BMT [149, 150].

The utility of in vitro susceptibility testing for filamentous fungi is poorly defined, as acquired resistance was uncommon and species identification was sufficient to guide antifungal therapy. However, both CLSI and EUCAST are developing and validating reference methods for in vitro susceptibility testing of *Aspergillus* and other conidium-forming fungi [151]. For *A. fumigatus* breakpoints have been proposed, while awaiting the recommendations by CLSI and EUCAST [152]. With the emergence of acquired resistance in *A. fumigatus* the need to determine the susceptibility in order to guide antifungal therapy has

increased. In general, voriconazole is appropriate first line therapy for most IFD in CGD, with the important exception of mucormycosis, for which lipid formulations of amphotericin are first line and posaconazole a possible oral substitute.

In 104 of the 116 cases information on prophylactic treatment was retrieved. Itraconazole or ketoconazole was prophylactically prescribed in 19 cases (18 %). In addition, 3 patients were still under antifungal treatment at the onset of a new IFD: 2 developed a *Scedosporium* breakthrough infection (*S. prolificans* during voriconazole and *S. apiospermum* during amphotericin B treatment) [107, 108] and one *Trichosporon* sp. was cultured during amphotericin B, caspofungin, and voriconazole treatment for active *A. nidulans* infection [113]. Twenty-eight patients received INF-γ (27 %), in almost half in combination with antifungal prophylaxis.

Interferon-gamma (INF-γ) is a macrophage-activating cytokine produced by T cells and natural killer cells and has a key role in the innate and adaptive host response against fungi [153]. A subgroup of variant X-linked CGD patients (i.e., with very low, but detectable, baseline superoxide-generating activity), who have splice site mutations, has been shown to be responsive to INF-γ [154, 155]. Treatment resulted in improved splicing efficiency and an increase in cytochrome b expression, allowing near normal levels of superoxide production and bactericidal activity of neutrophils and monocytes [156–158]. However, subsequent reports have not confirmed the enhancement of superoxide production as the principal mechanism of INF-γ activity in CGD individuals [159–161]. To date placebo-controlled studies are still limited and controversy remains about the routine prophylactic administration in CGD patients. INF-γ prophylaxis is offered only in selected CGD cases by most European physicians, while it is more universally prescribed in the USA and Japan [162, 163]. In contrast to the multiple studies confirming value for the use of INF-γ prophylaxis to reduce infections in CGD, there are no prospective data to show any benefit of INF-γ treatment during acute infection.

One of the cornerstones of clinical care for CGD patients is the lifelong prophylactic treatment with intracellular active microbicidal agents. After the introduction of antibacterial prophylaxis, fungal infections persisted with an incidence of 0.15 episodes per patient year [4]. In a prospective, open-label study with 30 patients, administration of itraconazole reduced the rate of *Aspergillus* infections to one-third in comparison with historical controls [164]. Gallin et al. [165] performed a randomized, double-blind, placebo-controlled crossover study in 39 CGD patients, finding only one serious fungal infection in the itraconazole group compared to seven cases in the placebo arm. Serious fungal infections developing during long-term itraconazole prophylaxis may well be due to molds that are resistant to itraconazole. Further studies are warranted to evaluate potential alternatives to the currently used antifungal prophylactic strategies.

3.8   Clinical Outcome

Unambiguous data on clinical outcome in accordance to the definition of complete response (defined as resolution of all clinical signs and symptoms attributable to IFD and complete or nearly complete radiological resolution) or a partial response (improvement or resolution of all clinical signs and symptoms attributable to IFD and ≥50 % improvement in radiological findings) were lacking and follow-up ranged from "still under treatment" to 9 years after treatment. At the time cases were published, the mortality rate related to the fungal infection was 26 % (Table 3.1). Mixed fungal infections (50 %) and mucormycosis (55 %) seem to be responsible for the highest mortality rates, followed by *Candida* spp. (40 %), *A. fumigatus* (30 %) and *A. nidulans* (27 %). However, the reported small numbers of the individual groups, the occurrence of particular species like *A. nidulans*, or severe clinical presentations of the more commonly encountered *A. fumigatus* may substantially influence these mortality rates. This underscores again the need of more prospective surveillance cohort studies of invasive fungal infections in this unique host.

3.9   Conclusions

To summarize our current understanding of fungal epidemiology and clinical management of invasive fungal disease in CGD patients we have to conclude that there is a paucity of data. Consequently, current management strategies may not be optimal to protect CGD patients from these life-threatening infectious complications.

Host defense mechanisms influence the manifestations and severity of infections; the clinical presentation of fungal disease depends on the patient's immune response. As a result, there are important clinical and diagnostic differences between IFD in CGD patients compared to patients with other immunocompromised conditions.

First of all, the CGD patient is a non-neutropenic host suffering from impaired phagocytic killing and a dysregulated inflammatory response. As a result, IFD can present with a huge variety of often non-specific symptoms, ranging from indolent and asymptomatic up to acute onset of hypoxia and respiratory failure, or isolated symptoms of specific organ involvement. Timely diagnosis of IFD in CGD requires a high degree of suspicion. This is in contrast to the neutropenic host, in whom the risk depends mainly on the duration of the neutropenia and in whom persistent febrile neutropenia is one of the early signs of a possible invasive fungal infection.

Secondly, invasive mould infections in neutropenic patients are characterized by hyphal angio-invasion, necrosis and paucity of inflammatory cells. In contrast, angioinvasion is not a feature of invasive mould infections in CGD. As a consequence, the characteristic radiologic halo-signs, air-crescents and cavities within areas of consolidation (one of the EORTC/MSG diagnostic criteria of invasive fungal infections) are typically lacking in CGD infections.

Furthermore, indirect laboratory markers of IFD, like the detection of *Aspergillus* antigen (galactomannan) in serum, have a low sensitivity in this patient group. Molecular diagnostic modalities, like the *Aspergillus* PCR, either have not yet been standardized or their sensitivity and specificity in CGD patients have not been

studied. As a result, the recognition and diagnosis of IFD in CGD patients is distinct from that in cancer and transplant patients.

Due to the rather long and obscure list of unusual invasive fungal infections in CGD patients, it is of the utmost importance to identify the causative fungus in a particular patient. In general, the clinical condition of CGD patients often can tolerate invasive diagnostic procedures and they should be performed to isolate the causative species, subsequently followed by targeted antifungal therapy. On the other hand, it is critical to keep in mind that the identification of an invasive infection in a previously healthy host is highly suggestive of CGD as the underlying disease.

IA caused by *A. nidulans* is virtually unique to CGD patients and needs more aggressive antifungal therapy than the more commonly encountered *A. fumigatus*. Invasive infections by *A. nidulans* are very seldomly reported in other immunocompromised patients and indicate a unique interaction between this fungus and the CGD host. The clinical entity of 'mulch pneumonitis' is a characteristic presentation of fungal pneumonia in the CGD patient in which multiple causative fungi may be recovered. The occurrence of infectious complications by Zygomycetes is mainly noted in the setting of inflammatory complications being treated with immunosuppressive drugs. *Candida* infections are less frequently seen but show an age-dependent clinical presentation; meningitis, fungemia and lymphadenitis with or without dissemination, were reported in neonates and young infants, and were not observed in older patients.

Details regarding strain identification and in vitro susceptibility testing are scarce in CGD patients. Accurate identification of the causative fungus to species level, due to a species dependent susceptibility to the currently used antifungal drugs for treatment and prophylaxis will definitely affect patient management. In vitro susceptibility testing may also have a role, but is as yet undefined.

Extensive and early surgery has been the cornerstone of treatment and amphotericin B was mostly used as the first-line drug. Since the introduction of the new triazoles, the occurrence of invasive fungal infections as a cause of death seems to decrease dramatically. It remains to be seen if the combination of an early diagnosis with an accurate specification of the causative fungus and subsequently directed antifungal therapy will improve outcome without the need for aggressive surgery. Adjunctive immunomodulatory therapy was used in about 25 % of the patients. The role for immunomodulatory agents in addition to antifungal therapy is not well studied. Due to lack of a profound understanding of the pathogenesis of invasive fungal infections in the CGD host, it is currently hard to define to what extent specific immunomodulatory therapies will be of benefit for this specific patient population.

Antifungal prophylaxis is not universally prescribed and has been associated with invasive infections caused by intrinsically or acquired resistant fungal organisms. However, since the introduction of azole prophylaxis the incidence of invasive aspergillosis seems to have decreased.

An infection-related mortality of 26 % was noted, but underestimation cannot be ruled out because of the huge variability in follow-up. Furthermore, large variability in the mortality was noted based on the clinical presentation and the causative fungus.

Management and outcome data should be gathered to guide optimal treatment decisions for future patients. At present, international and national databases are used to collect clinical data prospectively in children suffering from invasive fungal infections. Although not specifically designed for patients with CGD, it will be a first step to improve our knowledge about these devastating infections.

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Clostridium difficile in Children: A Review of Existing and Recently Uncovered Evidence

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Abstract

The clinical significance of the presence of *Clostridium difficile* in children's faeces remains uncertain using current diagnostic procedures. *Clostridium difficile* is a relatively common finding in infants with no symptoms of gastrointestinal disease, suggesting it may be an incidental finding and form part of the normal gut micro-flora in this age group. On the other hand, particularly in older children or those with significant co-morbidity, there are examples where *C. difficile* causes disease and exerts considerable morbidity and even mortality (*C. difficile* infection, CDI). Between these extremes lie a substantial group of children who have both diarrhoea and *C. difficile* in their stools but where the nature of the association is not clear: *Clostridium difficile* associated disease (CDAD). We review the significance of *C. difficile* in children presenting recently uncovered paediatric data from a large UK epidemiological study that informs some key unanswered questions.

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4.1 Introduction

The clinical significance of *Clostridium difficile* colonisation in children is crucial in determining testing and treatment policies. *C. difficile* is the leading cause of nosocomial diarrhoea in adults and one of the Healthcare Acquired Infections (HAI) specifically targeted by the National Health Service (NHS) in England [1]. Mandatory monitoring in the UK currently requires reporting of all diarrhoeal samples positive for *C. difficile* in those over the age of 2 years [2, 3]. *C. difficile* infection (CDI) in adults has been extensively studied and there are clear guidelines for its diagnosis and management [3–8]. The situation is much less clear in children because [9, 10] of the following issues:

a. ***Clostridium difficile* carriage rates are high in young children, especially infants:** Asymptomatic carriage of *C. difficile* in children is much more frequent than in adults. Carriage can be found in up to 64 % of neonates (under 4 weeks old), who appear to acquire *C. difficile* environmentally in the first few weeks of life [10]. Asymptomatic carriage is so high in infants under 1 year of age that testing for *C. difficile* is of questionable benefit [11]. Carriage declines with age, [12, 13] whilst the proportion with symptomatic disease increases to approach levels in adults (5–8 %, [10]).

b. ***Clostridium difficile* may be an incidental finding:** Acute diarrhoeal illness is common in young children, and due to a wide range of potential pathogens [12, 14–17], although in a substantial proportion no pathogen can be identified even after extensive testing for a range of bacteria, viruses and parasites. Given that so many children suffer diarrhoea without an identifiable cause, it is possible that even in those cases where *C. difficile is* identified, it is in fact not the causative agent but an incidental finding. It is even possible that colonisation with *C. difficile* may be protective [18, 19] against CDI.

c. **The relationship between prior antibiotic usage and *C. difficile* is considerably weaker in younger children:** Not only do many children with *C. difficile* in their stool sample not have a recent history of antibiotic exposure [10, 20, 21], but a significant proportion of them have concurrent infection with other pathogenic organisms [14, 20, 21]. Thus, two of the original diagnostic criteria for CDI in adults [6] would exclude a large proportion of symptomatic children with *C. difficile* positive stools. Although there are recent guidelines for diagnosing CDI in adults [7, 8], no equivalent guidelines exist for children, where the evidence base is much more limited.

Despite this, there is a consensus that *C. difficile* can cause serious disease in children [10, 22]. It is helpful to maintain a distinction between examples of CDI, where *C. difficile* is the accepted cause of children's symptoms, and "*C. difficile associated* disease" (CDAD) where the exact nature of the association between *C. difficile* and gastrointestinal symptoms remains unclear. Although variably defined, for example "gastrointestinal symptoms in a child with *C. difficile* toxin positive stools" [23], or "children with a clinical diagnosis of *C. difficile* infection on discharge who had *C. difficile* testing and antibiotic therapy for CDAD" [24], the incidence of CDAD and/or CDI in children appears to be on the increase, particularly in cases arising from the community [25]. Recent reviews have highlighted the importance of considering CDAD/CDI in children and outside the hospital setting [26–28]. Difficult questions are raised for clinicians faced with children suffering from diarrhoea [9, 10]:

1. When should *C. difficile* be considered as a cause for the child's symptoms and what is the appropriate testing strategy?
2. Should the presence of *C. difficile* in diarrhoea in children be treated, a*nd if so how*?

With current technologies, testing for *C. difficile* in children is controversial [11]. This is especially true for children under the age of 1, who are the most likely to suffer from diarrhoea, have the highest prevalence of asymptomatic carriage of *C. difficile*, and also form a group for whom the incidence of CDAD is increasing [23]. Devising the most appropriate policy has significant implications for all acute NHS Hospital Trusts in England, who face financial penalties if their

Table 4.1 Key features of different testing methods for *C. difficile*

	Tests requiring organism culture		Tests used directly on stool specimens			
	Selective culture	TGC	GDH EIA	PCRCDT	Toxin A/B EIA	CCA
Test target	Whole organism	Products/genes of isolate	Enzyme product	Toxin gene (s)	Toxin (s)	Toxin (s)
Detects						
Non-toxigenic *C. difficile*	+	−	+	−	−	−
Toxigenic *C. difficile*	+	+	+	+	±[a]	±[a]
Turnaround time	2–5 days	2–5 days	Minutes	1–3 h	Minutes	1–3 days
Performance in adults[c] Sensitivity		cf. CCA	0.80–0.97	0.87–1.00	0.31–0.99	
		cf. TGC	0.60–0.74	0.86–0.94	0.32–0.79	0.55–0.67
Specificity		cf. CCA	0.75–0.97	0.94–1.00	0.65–1.00	
		cf. TGC	0.76–0.95	0.94–0.97	0.84–1.00	0.98
Performance in children[d] Sensitivity[b]	0.75 (by EIA) 0.90 (by PCR)			0.95	0.35	
Specificity[b]	1.00			1.00	1.00	

[a] Tests are only positive if sufficient toxin present in faeces
[b] The "reference standard" was any stool specimen where at least four of the six tests used were positive (stool EIA, postculture EIA, stool PCRCDT for *tcdA*, postculture PCRCDT for *tcdA*, stool PCRCDT for *tcdB*, postculture PCRCDT for *tcdA*)
[c] Crobach et al. [7]; Tenover et al. [39]; Carroll [40]; Stamper [100]
[d] Luna [42]

rates of *C. difficile* infection fail to meet national targets under a system that has been criticised as unfair due to arbitrary thresholds [29]. This is a potential disincentive to test in populations expected to have higher prevalence of colonisation with *C. difficile*, or to improve the accuracy of testing strategies in ways that increase reportable rates of CDI [30]. Such conflicts can only be resolved by a better understanding of the significance of *C. difficile* colonisation and infection in children that can inform new guidelines.

4.2 The Complexity of Testing for *C. difficile* and Diagnosing *C. difficile* Infection

Conventional detection of *C. difficile* relies on selective culture of the organism from faecal samples or detection of toxin by cell culture cytotoxicity assay (CCA) or enzyme immunoassay (EIA) [4]. *Clostridium difficile*'s pathogenicity is associated primarily with expression of toxin B (cytotoxin), with the role of toxin A

(enterotoxin) being less certain [31, 32]. Not all strains are capable of producing toxin A [33, 34] so tests directed against this alone may miss CDI. Molecular assays based on amplification of *C. difficile* toxin gene sequences (PCRCDT) provide rapid and sensitive results but may not be as specific for CDI as phenotypic testing methods (e.g. CCA) which detect toxin production [35]. *Clostridium difficile* exhibits considerable genomic variation [36], allowing development of genetic typing schemes using techniques such as PCR ribotyping [37]. Genetic variation within the toxin genes may affect the performance of both phenotypic and genetic detection methods [38, 39]. Detection of *C. difficile* will therefore depend on the strain present and the test used (Table 4.1) [4, 7, 40]. A two-stage testing strategy has recently been implemented in the UK, [3] based on an observational diagnostic study in four UK laboratories [41] that confirmed that *C. difficile* toxin EIAs are not suitable as stand alone tests for the diagnosis of CDI or detection of *C. difficile*. The new UK recommendation contains a two test screening protocol comprising a

GDH EIA (or toxin gene PCR) followed by a sensitive toxin EIA. If the first test (GDH or toxin gene test) is negative, the second test (sensitive toxin EIA) does not need to be performed.

Unfortunately, nearly all of the studies comparing the performance of different testing strategies have been conducted in adults and the clinical significance of the results will be subject to different interpretations in paediatric and neonatal populations. One study that investigated both children and young adults is a recent prospective study from Texas that compared direct stool EIA toxin A/B and direct stool PCRCDT with toxigenic culture (TGC, testing for toxin with either EIA or PCRCDT after the organism has been cultured selectively from faeces) [42]. Stools from 96 patients (age 15 days–25 years, median 4 years) suspected of having CDAD were tested using EIA toxin A/B and PCRCDT, both before (direct) and after (TGC) isolation and selective culture. Although lacking an independent gold standard, the "reference standard" for calculating sensitivities and specificities was any stool specimen where at least four of the six tests used (stool EIA, postculture EIA, stool PCRCDT (*tcdA*), stool PCRCDT (*tcdB*), postculture PCRCDT (*tcdA*), postculture PCRCDT (*tcdB*)) were positive. Direct stool PCRCDT had the greatest sensitivity of all methods used (95 %, compared to only 35 % for EIA; specificity of both PCRCDT and EIA: 100 %). Interestingly, positivity rates for *C. difficile* have doubled (from ~8 to 16 %) since the introduction of PCRCDT as the standard testing strategy at this US institution (while the number of samples sent for testing has stayed the same).

4.3 Epidemiology of *C. difficile* in Children

Few published studies are well placed to establish when *C. difficile* should be considered the cause of a child's diarrhoea, or when it should be treated. We therefore reanalysed paediatric data from the English community-based Infectious Intestinal Disease Study (IIDS) [12, 43] which have not previously been reported within the peer

reviewed literature. The IIDS data is informative as the study attempted to: (a) test each sample for a range of viral, bacterial and parasitic pathogens to identify co-infection and the possibility that *C. difficile* is an "innocent bystander"; (b) use an appropriately sensitive testing strategy for *C. difficile* (including selective culture); (c) test both symptomatic and asymptomatic children to assess the association between *C. difficile* and symptoms; and (d) present data within clinically relevant age ranges. A second study (IID2) is currently in progress [44].

4.3.1 *Clostridium difficile* in Children in the Community

Several community-based studies have demonstrated that detection of *C. difficile* and/or *C. difficile* toxin is as common in asymptomatic children as in those suffering with diarrhoea (Table 4.2). Conducted between 1993 and 1996, the IIDS collected data and stools from over 6,000 cases of infectious intestinal diseases in children and adults, as well as from controls [12, 43]. The IIDS included two components: a community cohort recruited randomly from 70 General Practitioner (GP) practices across England and a GP-based case-control study which included subjects presenting spontaneously with symptoms of diarrhoea to one of 34 practices. All faecal samples were subject to extensive testing for a range of potential pathogens including *C. difficile*. Asymptomatic carriage of *C. difficile* was highest in infants under 1 year of age: 21 % in those recruited as part of the community cohort and 16.6 % in the age and sex-matched controls for those presenting to their GP with diarrhoea. Carriage rates in infants *with* diarrhoea were not much greater (28.6 % in the community cohort) or even less (7.2 % in infants presenting to their GP). 1 % of children aged 1–4 years of age had *C. difficile* in their faeces, and *C. difficile* was rarely found in children 5 years or older. There was no significant association of *C. difficile* with diarrhoeal symptoms for children under the age of 5 in either the GP study (derived odds ratio for diarrhoea if *C. difficile* positive 0.59; 95 % confi-

Table 4.2 Prevalence of *C. difficile* in children in the community: asymptomatic children compared to those with diarrhoea for different groups*

Group	Country	Dates	Age, years (median)	Prevalence (n)	Test Methods	Setting
Community Non-diarrhoea	U.K.	1993-6	<1 1-4 5-14	21.0% (19) 1.0% (98) 0% (72)	CCA (also selective culture (CCFA), TGC via Toxin A EIA, and PCR ribotyping)	Age- and sex-matched controls to community diarrhoea cohort (12)
	U.K.	1993-6	<1 1-4 5-14	16.6% (199) 1.0% (510) 0.6% (177)		Age- and sex-matched controls to GP presentations of diarrhoea, families not part of cohort (12)
	U.S.A.	1981	0.04 - 16	14.8% (135)	Selective culture (CCFA)	Outpatient attendees (45)
		2001-2	(1.27)	3.5% (484)	Toxin A/B EIA	Community (17)
	Japan	2004	<1 1 2 3 4 5	100% (12) 75% (20) 45.5% (11) 24% (25) 38.5% (13) 23.5% (17)	Selective culture, toxin gene typing (PCR), PCR ribotyping and pulsed-field gel electrophoresis	2 day nurseries and a kindergarten (13)
Community Diarrhoea	U.K.	1993-6	<1 1-4 5-14	28.6% (21) 0.9% (116) 0% (94)	CCA (also selective culture (CCFA), TGC via Toxin A EIA, and PCR ribotyping)	Diarrhoeal episode within community (asked to provide samples when have diarrhoea), recruited to cohort via GP practice lists (12)
	U.K.	1993-6	<1 1-4 5-14	7.2% (180) 1.7% (468) 0.6% (167)		Presentations to GP with diarrhoea, families not part of cohort (12)
	U.S.A.	1981	0.04 - 16	7% (171)	Selective culture (CCFA)	Outpatient attendees (45)
		2001-2	(1.27)	1.9% (431)	Toxin A/B EIA	Community (17)
		1998-2001	0.01-28.6 (1.3)	6.7% (688)	CCA	Paediatric emergency admissions (14)
		1998-01 2000-01	0.05-18.75 (1.61)	9.0% (89) 0% (15)	CCA	Private paediatric outpatient clinic Clinic serving mainly immigrants (15)
	Austria	2007	<5 5-19	5% (20) 6.5% (46)	Toxin A/B EIA and TGC (Toxin A/B EIA)	Presentations to GP with acute gastroenteritis (16)

*Where available, matching shades show data from the same study to facilitate comparison

dence interval (CI) 0.34–1.02) or the community cohort (odds ratio 1.21; 95 % CI 0.37–3.9).

Two other studies comparing *C. difficile* in symptomatic children with asymptomatic controls showed similar results. Boenning et al. [45] showed that the prevalence of *C. difficile* amongst paediatric outpatients was actually higher in those with non-diarrhoeal illnesses (14.8 %) than those with diarrhoea (7.0 %; Odds ratio 0.43, 95 % CI 0.20–0.92), and found no association with antibiotic exposure in the preceding month. More recently, Vernacchio et al. [17] carried out a prospective cohort study of healthy children in the community in which baseline normal stools were cultured and compared to those from the same children who subsequently developed diarrhoea during the study period. Of healthy baseline stools, 3.5 % contained *C. difficile* compared to just 1.9 % in diarrhoeal specimens (matched relative risk 0.54, 95 % CI 0.20–1.50). Contrast-

ing results were obtained from an earlier study of diarrhoeal outbreaks in community day centres [46], where rates of *C. difficile* isolation were greater amongst children with diarrhoea (57 %) than in their healthy classmates (9 %; OR 13.3, 95 % CI 3.5–51).

Other community-based studies have either tested only asymptomatic children [13] or children with diarrhoea [14–16], and therefore cannot provide comparative data regarding the association of *C. difficile* with symptoms. They nevertheless demonstrate that *C. difficile* is a relatively common finding (5–9 %, Table 4.2) in children presenting from the community.

Although the prevalence in different age ranges have varied between studies, a high prevalence of *C. difficile* has largely been found in young children and infants, with an average age in these studies typically 18 months or less. For example, in the recent US study [24] the mean

Table 4.3 Prevalence (%) of identified microorganisms in diarrhoeal samples from children in the community (IIDS GP data shown, cohort data not shown)

Putative pathogen		Setting							
Class	Organism	GP presentations [12] 1993–1996 (UK)			Paediatric outpatients[a] [15] 1998–2001 (USA)	Emergency department [14] 1998–2001 (USA)	Community diarrhoea [17] 2001–2002 (USA)	GP presentations [16] 2007 (Austria)	
		<1 year	1–4 years	5–14 years	0.05–18.75 years (1.6 years)	1.3 years	0.5–3 years (1.25 years)	<5 years	5–19 years
Bacteria	*C. difficile* (toxin)	7.2	1.7	0.6	9.0	6.7	1.9	5	6.5
	C. perfringens	4.0	5.6	4.5			2.3		
	Aeromonas spp.	10.3	4.3	7.2			1.0		
	Campylobacter spp.	2	5.4	11.3	4.1	1.5	0.7	0	4.3
	Pathogenic *E. Coli*								
	DAEC	3.6	2.9	2.3					
	AEEC	6.1	9.8	3.3					
	EAggEC	4.0	5.5	4.7			4.1		
	EPEC	0	0.2	0.5			12.9[b]		
	ETEC	0	0.5	0.5			0.2		
	EHEC/STEC	0	0.3	0.5	0	2.4	0.2	0	0
	Salmonella spp.	2.3	2.5	5.9	0	2.4	0	0	0
	Staphylococcus aureus	1.3	0	0					
	Yersinia	0.3	2.0	1.8	0.4	0.1	0.2	0	0
	Shigella spp.	0	0.3	0.4	0	0.9	0		
Viruses	Rotavirus	21.3	17.6	8.1	14.2	20.4	5.2	5	6.5
	Norovirus/SRSV	8.9	11.0	5.5			1.9	20	8.7
	Adenovirus	6.9	10.3	1.0	6.3	4.3	5.7	10	0
	Astrovirus	1.9	6.7	3.0	3.1	6.5	3.5	0	0
	Sapovirus/Calicivirus	5.8	3.8	0.5			3.0		
Parasites	*Giardia*	1.0	1.2	1.8		0.6	0.2	0	0
	Cryptosporidium	0.7	2.8	5.0		0.3	0.5	0	0
No pathogen identified		42.0	30.9	45.7	52	53	57.5	60	74

[a] Data from Site A only (private clinic)
[b] 'Atypical' EPEC

age was 15.2 (±7.5) months, with most children under the age of 3 years. In studies that report prevalence for infants separately this is universally higher than for older children.

A small study in Japan was notable for the particularly high prevalence of *C. difficile* in asymptomatic children at two day nurseries and a kindergarten [13]. All the infants tested had *C. difficile* in their faeces but carriage rates declined steadily with increasing age to less than 25 % by 5 years of age. Nearly all (21/22) of the *C. difficile* strains (by PCR ribotype and PFGE type)

isolated from environmental swabs at one nursery were identical to those found in the faeces of infants in that nursery, suggesting acquisition from the day-care environment.

Community onset diarrhoea is relatively common in young children and infants, with a range of potential pathogens other than *C. difficile*. Table 4.3 summarises data from five studies which carried out extensive testing for pathogens in children with community-acquired diarrhoea. A number of trends are revealed across the different settings, methodologies and even countries

where the studies were based. Despite the investigators' best efforts to test for a large range of pathogens, none could be identified in 30–75 % of the cases of diarrhoea. Viruses were the commonest identified pathogen in all studies, especially in infants and pre-school age children. Rotavirus was most frequently encountered (~14–20 %, except in an Austrian study, where there is routine vaccination against rotavirus [16]), followed by norovirus (where tested, ~9–11 %), adenovirus (~4–10 %) and astrovirus (~2–7 %). *Clostridium difficile* was the commonest identified bacterium in most studies. Other common isolates were *C. perfringens*, pathogenic *E. coli*, *Aeromonas* spp., *Salmonella* spp. and *Campylobacter* spp. (notably the latter two were more commonly isolated in older children).

A proportion of *C. difficile* positive children were co-infected with other known pathogens. In Klein et al.'s [14] study, 25 (6.7 %) of the 372 specimens that underwent extensive testing were positive for *C. difficile* toxin B, but nine of these had concomitant viral or bacterial pathogens. Only 4.3 % of children with diarrhoea had *C. difficile* as the only identifiable pathogen [14] and some common causes (e.g. norovirus) were not tested for, so it is possible that there may have been underestimation of co-infection with *C. difficile*. Denno et al. tested for the same range of organisms, and found one case of co-infection out of 75 children undergoing 'complete' analysis (one of the eight stools positive for *C. difficile* was also positive for adenovirus) [15]. Co-infection is also common in hospitalised children, with other pathogens reported in 36 % [21], 44 % [20] 27 % [47] and 23–38 % of *C. difficile* culture positive stools [48]. In three recently reported cases of severe CDAD in children, it was noted that two had co-infection with viruses (rotavirus and calicivirus), although these two also had underlying chronic medical conditions (Hirschsprung's disease and Down's syndrome) [49].

4.3.1.1   Explanations for High Levels of Asymptomatic Colonisation

The high rates of carriage of *C. difficile* may be explained by a lack of 'colonisation resistance' in infants as the intestinal micro-flora is in the process of becoming established [50], but this cannot explain the absence of symptomatic disease (CDI). Asymptomatic carriage does not simply occur because the strains of *C. difficile* present are non-toxigenic, for most of the studies discussed previously demonstrated the presence of toxin in faeces from asymptomatic children. One hypothesis is that the strains typically found in children have reduced virulence compared to those in adults. Factors other than toxin expression, such as those influencing adherence and intestinal colonisation, are also recognised contributors to virulence and may play a part in childhood disease [51].

The hypervirulent strain of *C. difficile* associated with outbreaks of severe CDI in adults (PCR ribotype 027) has greatly increased *in vitro* levels of expression of *tcdA* (16-fold) and *tcdB* (23-fold) [51]. This strain was responsible for a high proportion (19.4 %) of *C. difficile* toxin positive stools in symptomatic children in a recent study in the USA [52]. A study of hospitalised but asymptomatic children in Thailand demonstrated relatively high carriage rates for *C. difficile* (11.0 % of infants, and 21.1 % of 1–11 year olds) but low rates of toxin A gene detection (0.9 % of infants and none of the older children; toxin B not tested) [53]. 87.2 % of the strains isolated in asymptomatic children in the Japanese day-care study were also non-toxigenic (*tcdA*−/*tcdB*−) [13]. Unfortunately neither study examined rates of carriage or toxin expression in symptomatic children for comparison. A small study in Brazil attempted to compare strains of *C. difficile* in hospitalised children with acute diarrhoea with asymptomatic children recruited from day-care centres, but none of the controls were culture positive for *C. difficile*. Nine out of the ten strains isolated from symptomatic patients were toxigenic: six were *tcdA*+/*tcdB*+ and three were *tcdA*−/*tcdB*+ [54].

Only the IID study provides direct data comparing *C. difficile* colonisation and toxin B detection for cases of diarrhoea and asymptomatic controls [12]. Below 2 years of age, children presenting to their GP with diarrhoea had slightly higher rates of *C. difficile* colonisation (24 % of 374 cf. 19.5 % of 385 cultured) but lower rates of toxin B detection than asymptomatic age- and sex-matched controls (4.3 % of 391 cf. 9.0 % of 423 tested). For children 2 years or older, con-

Table 4.4 Prevalence of *C. difficile* in hospitalised children for different groups*

Group	Country	Year	Age Years (median)	Prevalence (n)	Test Method	Setting
Hospital Diarrhoea	Germany	1989	**0-1** **>1**	**~30%** **~5%** **(418)**	Culture and Y-1 cell culture (Toxin B) and rabbit ileal loop test (Toxin A)	Hospitalised children (55)
	Canada	1991-99	**0.03-17 (1.3)**	**18.0%**	Toxin A EIA	Paediatric and Women's Hospital (56)
	USA	1998-99 2001-03	**(1.3)** **(2.4)**	**26% (676)** **(Toxigenic: 15%)** **49% (301)** **(Toxigenic: 14%)**	Selective culture (CCFA) and TGC (PCRCDT), PCR fingerprinting	Stand alone paediatric hospital Paediatric department within general hospital (48)
	Turkey	2001	**0.08-13** **(Mean 3.2)**	**22% (100)**	Toxin A/B EIA	Inpatients treated with antibiotics with diarrhoea (47)
Hospital Non-diarrhoea	Germany	1989	**0-1** **>1**	**~30%** **~5%** **(348)**	Culture and Y-1 cell culture (Toxin B) and rabbit ileal loop test (Toxin A)	Hospitalised children (55)
	Thailand	1998-99	**0-1** **1-11yrs**	**11.9% (235)** **21.1% (76)**	CCFA culture, PCR *tcdA* and PFGE analysis for type	Hospitalised children (53)
	Turkey	2001	**0.08-13** **(Mean 4.1)**	**10% (50)**	Selective culture (CCFA) and Toxin A/B EIA	Inpatients treated with antibiotics but asymptomatic for diarrhoea (47)

* Matching shading shows data from the same study to facilitate comparison

trols had lower rates of both toxin B detection (0.2 % of 1,616 cf. 1.1 % of 1,866 tested) and *C. difficile* colonisation (0.4 % of 1,613 cf. 1.0 % of 1,845 cultured). These data suggest that above the age of 2 years, rates of colonisation drop dramatically, and toxin production is more likely to be associated with disease.

4.3.2 *Clostridium difficile* in Hospitalised Children

The prevalence of *C. difficile* in hospitalised children is higher than in the community but appears similar in children with and without diarrhoea (Table 4.4). Karsch et al. [55] found high rates of carriage, particularly in infants (30 %), with most isolates producing toxin (82 % toxin A and 43 % toxin B), but no significant difference between symptomatic children and controls, and no clear association with previous antibiotic therapy. A prospective study in Denmark also showed significantly higher isolation of *C. difficile* in infants and no relationship to antibiotic exposure ($p<0.001$), but it was the only identified pathogen in 12 % of children of all ages with acute gastroenteritis, and this was significantly ($p<0.01$) higher than for asymptomatic controls [21]. As 78 % of the positive

cultures were obtained within 2 days of admission this suggests acquisition in the community rather than nosocomial infection. A retrospective cohort study in the US reported that 26 % of cases of CDAD occured in infants [24], but it remains unclear whether *C. difficile* was truly the underlying cause. Of the 56 % of specimens from paediatric inpatients in Canada with nosocomial diarrhoea where a pathogen was identified, most were viruses (38 % of episodes, viral diagnosis typically in younger children with mean age 0.8 years) [56]. *Clostridium difficile* was identified in 18 % of all episodes (mainly in older children, mean age 3.9 years). A retrospective case control study in Canada was unable to demonstrate any difference in clinical characteristics between infants with *C. difficile* toxin in their stool and those without toxin present, nor could it identify a significant treatment effect of metronidazole [11]. Colonisation with *C. difficile* was relatively high at two US institutions (49 % and 26 %), but toxigenic colonisation was less common (14 % and 15 %), and many isolates were unique (92 % within the general hospital), indicating that they did not arise from a common source [48]. Underlying medical conditions and exposure to two or more antibiotics were associated with increased toxigenic strain colonisation. In Turkey, although *C. difficile* toxin was found more frequently in

hospitalised children with nosocomial diarrhoea (22 %) than asymptomatic controls (10 %), this was not significant (odds ratio 2.54, 95 % CI 0.90–7.17) and co-infection was found in six cases (27 %) of CDAD (rotavirus was the only viral pathogen tested) [47]. Children with CDAD were older (mean 5.4 years) than those asymptomatic controls with *C. difficile* toxin in their stools (all under 2 years).

4.3.3   *Clostridium difficile* in Neonates

Neonates acquire *C. difficile* from the environment, resulting in high rates of carriage within the first few weeks of birth [57–60]. In one study, no neonatal faeces cultured *C. difficile* on day 1 after birth but 17 % did by day 4, and most strains were toxigenic (58–65 %) [60]. None of the maternal rectal swabs and only one vaginal swab cultured *C. difficile,* whereas 13 % of environmental samples did and these were all of the same strain (matching 11 of the 31 neonatal strains typed). On one neonatal intensive care unit (NICU), *C. difficile* acquisition reached 33 % after 2 weeks with all cultures toxigenic [61]. In common with previous studies [58, 59], there was no association between *C. difficile* acquisition and gastrointestinal symptoms. In another NICU, 90 % of samples cultured *C. difficile* after only 6 days, and although toxin was detected directly in only 36 % of these, 94 % of the isolates were found to be toxigenic *in vitro* [57]. One small study suggested that neonates with toxin A positive stools are likely to experience increased numbers of days with frequent and abnormal stools [62], but most demonstrate asymptomatic carriage of *C. difficile* in neonates, even when a high proportion of stools test positive for toxin.

4.4   Burden of CDAD in Children

The high asymptomatic prevalence of *C. difficile* in young children initially led to the conclusion that CDI was not a problem in this age group [45, 63]. However, several studies have suggested that CDAD (and by implication CDI) is an increas-

ing problem in children. A retrospective cohort study in the USA showed that from 2001 to 2006 the incidence rate of CDAD (defined as "clinical symptoms, such as diarrhoea or bloody stools, in a patient whose stool specimen tested positive for *C. difficile* toxin") amongst outpatients increased by 11 % (from 1.24 to 1.38 cases per 1,000 visits) [23]. The incidence of CDAD in patients attending the emergency department increased 2.5 fold, largely due to an increase in community-associated CDAD (from 0.84 to 2.04 cases per 1,000 visits), while inpatient incidence of CDAD decreased over the same period [23]. In another study the annual incidence of CDAD (identified by the combination of discharge diagnosis of CDI, positive test assay for CDI and treated with antibiotics against CDI) increased from 2.6 to 4.0 cases/1,000 admissions over the same time period [24]. Hospitalisation rates for children with CDAD almost doubled between 1997 and 2006 [64]. Complications of CDAD can be severe, including pseudomembranous colitis [22, 49, 65, 66], rectal prolapse [67], osteomyelitis [68] and reactive arthritis [69, 70].

4.5   When to Consider CDAD and How to Test for it

The new UK Department of Health testing algorithm [41] contains a two test screening protocol comprising a GDH EIA (or toxin gene PCR) followed by a sensitive toxin EIA. If the first test (GDH or toxin gene test) is negative, the second test (sensitive toxin EIA) does not need to be performed.

4.5.1   Children Without Co-morbidities

Because the prevalence of *C. difficile* in children is so dependent on age it might be more appropriate to tailor the testing and management strategy accordingly (Table 4.5). Given the very limited data on the performance of different testing strategies in children (Table 4.1), adult guidelines may eventually be shown to perform poorly in children, where PCRCDT might be

Table 4.5 Suggested investigation and management of children with suspected *C. difficile* infection based on current evidence

Age	Rationale	Management
Neonates (0–4 weeks)	High incidence of carriage, true CDI very rare/indeterminate	Do not test for *C. difficile*. If very unwell, treatment for necrotising enterocolitis will include cover for *C. difficile*
Infants (4 weeks–1 year)	Relatively high prevalence of asymptomatic carriage of *C. difficile* and diarrhoeal illness common (likely CDAD rather than CDI)	UK 2012: GDH EIA (or toxin gene) followed by a sensitive toxin EIA[a]. If strong clinical suspicion of CDI, test faeces for *C. difficile* and for common viral and bacterial pathogens. Where community acquired and treatment *not considered urgent*, treat for CDI only if GDH EIA (or toxin gene) *and* sensitive toxin EIA positive *and* viral and other bacterial testing negative. If hospital acquired or urgent treatment considered necessary, treat for CDI if GDH EIA (or toxin gene) *and* sensitive toxin EIA positive
Pre-school (1–4 years)	Incidence of community acquired CDAD declines with age; incidence of CDI increases above the age of 2 years. Significant risk of viral pathogens causing disease	
School age (5–18 years)	Community acquired diarrhoea; risk of CDI increases with increasing age, also risk of other bacterial and viral pathogens. Hospital acquired diarrhoea: CDI incidence similar to adults: investigate and manage as for adults	

For a child presenting with a mild or recent onset diarrhoeal illness then this is likely to be viral and self-limiting: *no testing is indicated* and the child should be managed supportively according to recent NICE guidance [71]

For a child with a significant chronic disease (IBD, cystic fibrosis, cancer, HIV) and significant diarrhoea there is a higher risk of CDI requiring treatment. PCRCDT is the most appropriate first line or only test for paediatric samples where available. *Treat for CDI if PCRCDT positive.* (see footnote[a])

Secondary care management *where diarrhoea is severe or protracted* (>*7 days*)

[a] Current paediatric evidence suggests a more prominent role of PCRCDT may be justified in future testing strategies

considered a more appropriate formal second line test after GDH EIA due to its rapid turnaround time and good correlation with toxigenic culture (Table 4.1).

Infants have high asymptomatic prevalence, so testing stools for *C. difficile* is not recommended unless illness is severe and there is a high level of clinical suspicion [71]. Serious cases of CDI (pseudomembranous colitis) have been reported in infants, but have generally been associated with other conditions such as prematurity, Hirschprung's disease, obstruction or necrotising enterocolitis [65, 72, 73], and the role of *C. difficile* in the pathogenesis has not been confirmed. These reports suggest symptomatic infants should only be tested for *C. difficile* in carefully selected circumstances. Other organisms may also be responsible for disease and viral testing in particular should be undertaken alongside testing for *C. difficile*.

It would be helpful to identify reliable predictors for development of CDI in children. Despite the conflicting evidence for an association between antibiotic usage and CDI in children (against association: [11, 20, 21, 45, 55, 74, 75]; for association: [14, 47, 48, 63, 76–79]), severe diarrhoea in the context of recent antibiotic therapy is likely to remain one such predictor that clinicians will use.

4.5.2 Children with Co-morbidities

Certain co-morbidities are associated with higher rates of *C. difficile* colonisation, and some of these children may be at particular risk of CDI: inflammatory bowel disease (IBD), Hirschprung's disease, cystic fibrosis, cancer patients and organ transplant recipients.

The prevalence of *C. difficile* is significantly greater in children with IBD compared to controls (indicating an increased risk of colonisation) and also greater in children with IBD experiencing active disease compared to those with inactive disease (indicating a potential role of the organism in the symptom exacerbation) [80]. It is important to use an appropriate testing strategy to prevent missing CDI in the context of inflammatory bowel disease, as the diagnosis may be missed in up to 41 % of IBD patients if a single-toxin

assay is used [81]. Such errors could lead to misattribution of symptoms to an exacerbation of underlying IBD and result in inappropriate treatment, potentially even colectomy. Steroids given to treat IBD without appropriate antibiotics to treat CDI are likely to exacerbate symptoms rather than resolve them [82].

Case reports of severe CDI associated with Hirschsprung's disease suggest that this may form another group of children at increased risk [66, 83]. Increased *C. difficile* was found in Hirschsprung's patients with enterocolitis compared with asymptomatic Hirschsprung's patients or healthy controls [84].

Carriage rates of *C. difficile* in patients with cystic fibrosis have been reported to be 22–46 %, double that of control patients receiving antibiotics [85–87]. These high carriage rates were described in older patients (median age 18.5, youngest 15 years) in the absence of symptoms of diarrhoea or abdominal pain, despite the presence of toxigenic strains [87]. Nevertheless, severe cases of CDI do occur in children with cystic fibrosis, emphasizing the importance of considering the diagnosis [88]. Patients may present atypically, without watery diarrhoea but rather abdominal distension and reduced bowel motions, risking confusion with faecal impaction or meconium ileus equivalent [89, 90]. There also appears to be a greater risk of CDI following lung transplantation for cystic fibrosis, with patients experiencing a fulminant course resulting in high mortality [91, 92].

Cancer patients are thought to have increased carriage of *C. difficile* due to their chemotherapy treatment and increased exposure to antimicrobials [74, 77]. Most reported cases have been associated with haematological malignancies, but a recent Italian study demonstrated that 6 % of children with solid tumours had *C. difficile* toxin A in their stools and gastrointestinal symptoms, with three out of nine of these being under 1 year old [93]. An earlier prospective study of oncology patients demonstrated higher rates of toxin detection in asymptomatic (19 %) than in symptomatic (8.7 %) children, questioning the significance of *C. difficile* as a pathogen in this patient group [74].

4.6 Treatment Strategies

If *C. difficile* is the only pathogen identified and seems the most likely cause of disease in a child over the age of 1 then treatment should be considered [10]. In adults, where exposure to broad spectrum antibiotics is a recognised trigger for CDI, cessation of the antibiotic, if clinically possible, is an important step. As previously discussed, the association between antibiotic use and CDAD in children is much weaker, but it is sensible to discontinue broad spectrum antibiotics where possible. Oral metronidazole and, if this fails, vancomycin are the standard treatments for adults with severe CDI requiring treatment and are also used in children. Up to 25 % of patients experience a further episode of CDI within 2 months, and 50–65 % of these will suffer from further recurrences [94].

More recently, interest has moved to alternative treatment strategies for CDI, in particular to prevent recurrence of disease [10, 95]. Alternative strategies may be divided into those that attempt to re-establish colonisation resistance (use of probiotics, faecotherapy), direct chemical neutralisation of the toxins (toxin binding by ion exchange resins and polymers) and immunotherapy (passive immunoglobulin therapy) [94, 95]. Unfortunately, few randomised controlled trials have been conducted in adult populations, and none have been done in children. A recent Cochrane review [96] found little evidence that probiotics were useful in adults, identifying only one study that showed a beneficial effect when added to antibiotic therapy [97]. Extrapolation of such data to children is problematic given that in a child's early years the natural intestinal flora is different from adults and evolves over time. None of the alternative strategies explored so far has demonstrated sufficient success to recommend incorporation into general clinical practice [95].

Table 4.6 Outstanding questions for research

Are children suffering with CDAD colonised with different strains of *C. difficile* from those found in asymptomatic, age-matched controls?
What are the virulence factors other than toxin expression that influence pathogenicity in *C. difficile* and how do these explain the varying prevalence of symptoms with the age of the host?
Are there markers that correlate more closely with disease than toxin expression? Such markers might be specific to the strain of *C. difficile* present (e.g. other virulence genes) or the host specific response to infection (e.g. cytokine profile)
How do the new 'gold standard' tests for diagnosing *C. difficile* infection in adults perform in children at different ages?
What relationships exist between *C. difficile* and other members of the gut micro-flora, in both health and disease?
How common is co-infection in CDAD in children?
Are other bacterial or viral pathogens a risk factor for the development of CDI or *vice versa*?
Is colonisation with specific strains of *C. difficile* protective against other pathogens in children?
What factors underlie geographical variations in the prevalence of *C. difficile* colonisation and infection in children?

4.7 Conclusions

Clostridium difficile is a relatively common finding in the faeces of infants under 1 year of age, and is very unlikely to signify disease, even when toxins are produced. Children with diarrhoea may have *C. difficile* in their stools, but viruses are a more likely cause of symptoms. It is not possible to identify the aetiological agent in a large proportion of childhood diarrhoea, but the disease is typically self-limiting and requires only supportive care [71].

CDAD appears to be increasing in children and should be considered as adult-type CDI whenever symptoms are particularly severe, protracted or the child belongs to a known at risk group such as inflammatory bowel disease or cystic fibrosis following lung transplantation. The use of predictors for CDAD such as recent antibiotic usage or prior hospitalisation is generally unhelpful in children, where these associations are much weaker and colonisation appears to occur in the community.

Few diagnostic tests in routine clinical use for *C. difficile* infection have been evaluated in children, where the true sensitivities and specificities are likely to be different from adult populations. The evidence on which to base decisions of when and how to treat CDAD in children remains limited and many important, interrelated research questions are yet unanswered (Table 4.6).

4.8 Search Strategy and Selection Criteria

This review was prompted by re-appraisal of data contained in a large, UK government-funded investigation report (The Infectious Intestinal Diseases (IID) Study in England [12]) in the light of a growing appreciation that *C. difficile* in children differs substantially from adults [9, 10, 98]. The IID study produced substantial data on the incidence of *C. difficile* in children in the community not contained in the original journal article [43], which combined data for children and adults. Other data and references were obtained by searching PubMed using "infant", "child(ren)", "p(a)ediatric" and "*C. difficile*" as search terms. Retrieved titles and abstracts were screened and full text versions obtained of suitable articles in English. Where necessary, odds ratios and confidence intervals were derived from original data presented in the manuscript by a standard method [99].

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Diarrhea Among Children in Developing Countries

James P. Nataro

Abstract

Diarrhea continues to stand among the most important causes of global morbidity and mortality in children under 5 years of age. Although the introduction of oral rehydration and other case-management strategies have reduced acute diarrhea fatalities, many of the survivors develop persistent diarrhea and/or deficiencies of growth and cognition. Thus understanding the true global burden of diarrhea requires attention to acute diarrhea as well is its sequelae. To understand the etiology of moderate to severe diarrhea among children in high mortality areas of sub-Saharan Africa and south Asia we performed a comprehensive case-control study of children under 5 years of age at seven sites. Each site employed an identical case-control study design and each utilized a uniform comprehensive set of microbiological assays to identify the likely bacterial, viral and protozoal etiologies. Results of the studies will inform diarrhea prevention and management efforts worldwide.

5.1   Introduction

Diarrhea as a clinical complaint has been with hominids long before our species emerged, but in man's most natural state, infectious diarrhea may not have been a major health problem. Among small bands and tribes, infectious agents could have spread through the local population, engendered widespread immunity, then submerged to a subclinical existence, or perhaps failed to be sustained at all. With the rise of human cities, however, enteric pathogens may have found an opportunity for persistence, given continual replenishment of susceptible individuals, along with new opportunities provided by sewage systems and close proximity to domesticated livestock.

But historical records suggest that diarrhea emerged as a global scourge with widespread confluence of human populations brought about by colonization and frequent inter-continental transportation. Cholera spread dramatically across the globe in the ninteenth century in a series of pandemics [1]. No civilized area

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remained untouched. At the same time, cholera in the headlines drove breakthroughs in science and medicine from which we daily benefit. John Snow's investigations into the source of London cholera epidemics founded the science of epidemiology [2]. Robert Koch's seminal investigations, coupled with the infamous opposition by von Pettenkofer [3] and others, drove the final nails in the coffin of the miasma theory and established modern microbiology.

Realization that cholera derived from a microorganism spread through sewerage eventually drove development of modern urban sanitation systems. Sanitation finally stemmed the tide: deaths due to enteric diseases in industrialized cities declined dramatically around the turn of the twentieth century, long before we knew of antibiotics or oral rehydration therapy [4]. Unfortunately, however, while cities in the industrialized world reaped the benefits of cleaner living, the sprawling metropolae of the under-developed world continued to provide ripe opportunities for propagation of enteric diseases. In many parts of the world, this heritage persists.

5.2 Toward Effective Control of the Global Diarrhea Burden

The legacy of cholera also provided the first breakthrough in diarrhea therapy. In the 1950s and 1960s, physiologists described the phenomenon of coupled sodium-glucose co-transport across the mammalian intestine [5]. In the presence of glucose, animal perfusion models showed dramatically improved sodium uptake. Capt. Robert Phillips and others quickly exploited this fundamental breakthrough [6]. Working in Bangladesh (while others in Calcutta were making similar strides), Phillips' team demonstrated that stool output of cholera patients was substantially reduced when oro-gastric sodium chloride infusions included glucose [7]. With astonishing rapidity, this observation led to the miracle of oral rehydration therapy (ORT) [8].

ORT was propagated across the face of the globe throughout the decades of the 1970s, 1980s and 1990s, with remarkable success. Everywhere ORT reached, mortality rates from dehydrating diarrhea declined. Global data reflected the local effects. From an estimated 5 million deaths per year attributable to diarrhea among children under 5 years globally in 1980, this rate fell steadily to below 2 million, and data from the Child Health Research Group (CHERG) place the number of diarrhea-related deaths at 1.32 million in 2008 [9]. This still represents ca. 18 % of all deaths among children under 5 years (Fig. 5.1).

After rapid decline soon after the introduction of ORT, the rate of decline of diarrhea-related deaths has plateaued. Several causes can be postulated. First, the vigor accompanying early ORT implementation programs could not be sustained indefinitely, in view of new public health priorities. In addition, many enteric infections may be intrinsically refractory to ORT, or perhaps their pathophysiologic assault may involve derangements other than dehydration. Studies focused on the etiologic agents of childhood diarrhea now suggest that the story is complex.

5.3 Pathogenesis of Infectious Diarrhea

The fundamental derangement in human diarrhea is the reprogramming of the gastrointestinal tract from a net absorptive organ to one that eliminates feces with abnormally high water content. Molecular mechanisms may involve up-regulation of secretion channels and pumps, reduction of absorptive functions, and/or erosion of intestinal barrier function. Remarkably, pathogenesis research suggests that most enteric pathogens effect more than one (quite often all three) of these assaults. Enteropathogenic *E. coli* (EPEC), a common pathogen of infants and toddlers in developing countries, provides a prominent example (reviewed in references [10–13]) (Fig. 5.2). EPEC attaches tightly to the brush border of small intestinal enterocytes, and injects a set of protein toxins directly into the cellular cytoplasm. The net effects of these toxins are astonishing, and only partly understood [14]. A subset of the proteins trigger signal transduction pathways in the afflicted cell, resulting in

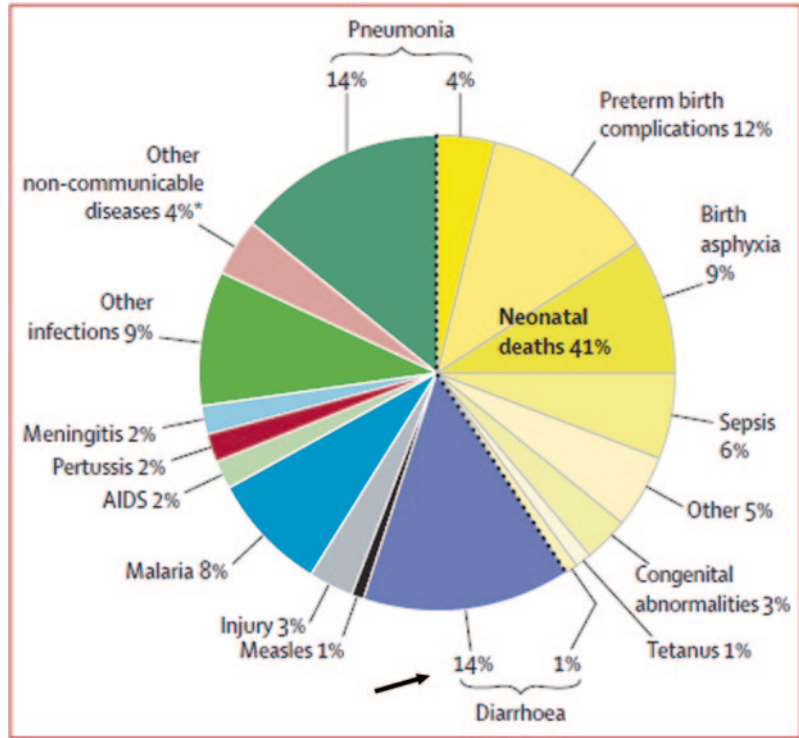**Fig. 5.1** Major causes of death among children less than 5 years of age worldwide, 2008. (From [9])

Fig. 5.2 Pathogenetic effects of enteropathogenic *E. coli* infection on small bowel epithelial cells. Attachment of the pathogen to the apical membrane of enterocytes results in decreased absorptive and enhanced secretory mechanisms, along with impairment of normal barrier function. (Adapted from [13])
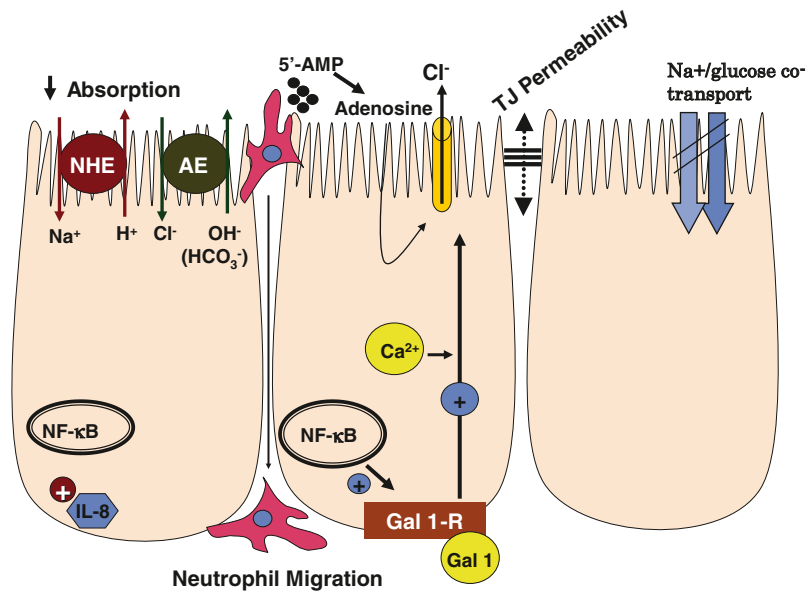
release of pro-inflammatory cytokines, including the chemokine IL-8, though pro-inflammatory responses are finely modulated [12]. Release of these cytokines results in recruitment of leukocytes to the tissue, leading to tight junction opening and tissue damage. Emerging evidence also suggests that some of the EPEC effector proteins may have direct pathologic effects on cellular absorptive and secretion channels in the apical membrane. Perhaps more remarkably, several proteins act in synergistic fashion to open tight junctions and stimulate leakage of fluid via the
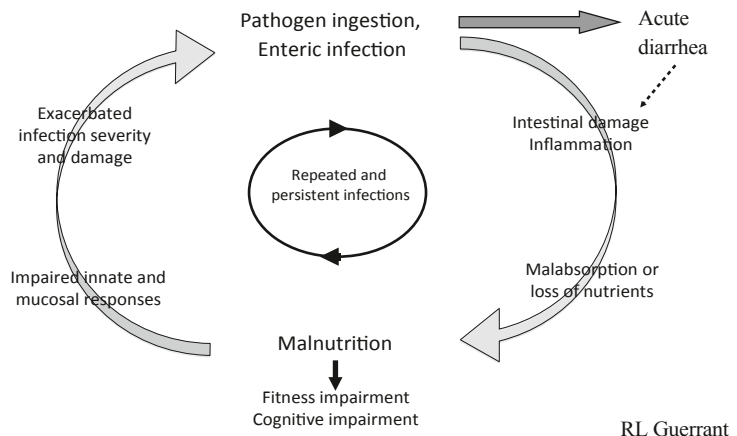
intercellular junctions [13]. One of the more intriguing effects of EPEC intoxication is the inhibition of the sodium-glucose co-transport apparatus [15]. It can be envisioned that damage to the epithelial layer, loss of absorptive driving force, and reduced function of the sodium-glucose contransporter could render EPEC infection relatively resistant to the effects of ORT. This hypothesis requires clinical validation, but there can be little doubt that the impact of some enteric pathogens extends well beyond loss of water and salts.

5.4   Diarrhea, Growth and Cognition

The notion that diarrhea does more than simply dry out the host has gained increasing support from epidemiologic and clinical studies. Studies in Fortaleza Brazil and elsewhere show clearly that patients with diarrhea persisting longer than 7–14 days demonstrate shortfalls in growth and cognitive development, as well as increased mortality that may be due to factors other than diarrhea per se. Guerrant and others have accordingly suggested that enteric infection may trigger a vicious cycle in which damage to the intestinal mucosa leads to malabsorption of nutrients that are required for growth, cognitive development, maintenance of gut integrity and immune function [16, 17]. This relationship has complex and potentially devastating implications. Fig. 5.3 illustrates the diarrhea vicious cycle: infection leads to malabsorption, which predisposes to further infection. Each individual infection exacts its toll in terms of growth and development; eventually, the ability of the child to "catch up" is lost. The effect has been documented since the 1970s, starting with the famous studies of Leonardo Mata in Guatemala [18]. In growth curves of children in Mata's study villages, he observed growth flattening accompanying each infection, followed by catch up growth that is increasingly meager as the child ages. The result is a child who is stunted, and likely abnormal in many more subtle ways. And as we rescue more and more of the word's children from acute diarrhea-related death, this silent toll of diarrhea and malnutrition may emerge as the principle threat of enteric infection on human health.

5.5   The Global Enteric Multisite Study (GEMS)

The urgency to understand the persistence of diarrhea-related death among the world's poorest children has motivated the Bill & Melinda Gates Foundation to embark on the largest diarrhea case-control study every attempted. The study, coordinated by Dr. Myron M. Levine at the University of Maryland School of Medicine, has implemented an identical case-control design at seven sites in sub-saharan Africa and south Asia (Table 5.1). For a period of 3 years, the study enrolls children under 5 years of age who pres-

Table 5.1 Sites represented in the global enteric multi-site research study

Country	Site	Pincipal investigator	Characteristics
The Gambia	Medical Research Center, Basse	Richard Adegbola	Rural, low HIV prevalence
Mali	Center for Vaccine Development, Bamako	Samba Sow	Urban, low HIV prevalence
Kenya	Centers for Disease Control, Kenya Medical Research Institute	Robert Breiman	Rural, high HIV prevalence
Mozambique	Centro de Investigaçao em Saude da Manhiça	Pedro Alonso	Suburban, high HIV prevalence
Pakistan	Aga Khan University Hospital, Karachi	Anita Zaidi	Urban coastal
India	National Institute of Cholera and Enteric Diseases, Kolkata	Sujit Bhattacharya	Urban slum
Bangladesh	International Center for Diarrheal Disease Research, Dhaka. (Mirzapur field site)	ASM Faruque	Rural and town

ent to sentinel health centers with a complaint of acute moderate-to-severe diarrhea, defined as diarrhea within 7 days of onset accompanied by clinical dehydration, fecal blood, need for intravenous hydration, and/or admission to a medical facility. For each of the subjects enrolled, a matched control is recruited from the subject's village.

Each GEMS case and control is studied in comprehensive fashion. A detailed questionnaire is administered to elucidate risk factors for enteric infection. Precise anthropometric data are acquired. A stool sample is collected and analyzed comprehensively for the major enteric pathogens, using validated microbiological methods. A household visit is conducted at 60 days post-enrollment for both cases and controls to ascertain survival, length of diarrhea, and growth during the follow-up period. Results of the study are expected in 2012.

5.6 The Future of Diarrhea Control

Future diarrhea prevention and therapy strategies must remain focused on the last ca. 1 million diarrhea-related deaths among children under 5 years of age, but also on diarrhea-related morbidities. The most fruitful approach to avoiding diarrhea-related deaths will require a multi-pronged approach. Rotavirus remains the most common cause of dehydrating diarrhea, and implementation of the new rotavirus vaccines has become an urgent global health priority. Successful deploy-

ment of the rotavirus vaccines to the world's poorest will save many thousands of lives, though the precise impact will depend on complex epidemiologic and political factors [19, 20]. Beyond the rotavirus vaccine, it is essential to elucidate other specific etiologic agents that may be overrepresented among fatal cases. These pathogens may become amenable to vaccines or control strategies targeted at their modes of transmission. The GEMS study should provide vital illumination of these important microorganisms. GEMS may also reveal information to guide sanitation and hygiene programs, particularly important in areas where pathogen-specific interventions are impractical.

Along with prevention of diarrhea-related deaths, we must become increasingly attuned to the silent morbidity attending diarrhea. Provision of zinc supplementation to children with diarrhea has already been shown to diminish the duration of diarrhea episodes. Providing anticipatory zinc to at risk populations may provide significant benefits in terms of diarrhea related morbidity and mortality [16, 21–31]. Several studies have examined the provision of zinc along with other nutrients (e.g. vitamin A), and inclusion of zinc in oral rehydration salts may be a practical way to target populations in greatest need [21, 23, 25, 30].

Understanding the effects of diarrhea on human cognition will become increasingly important as we prevent death and growth shortfalls. The studies in Fortaleza reveal not only that children with diarrhea are more likely to mani-
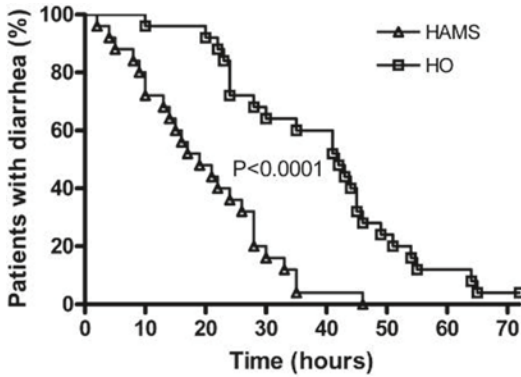
Fig. 5.4 Compared with standard ORS, high amylase maize starch (HAMS-ORS) reduced diarrhea duration by 55 % and significantly reduced fecal weight after the first 12 h of ORS therapy in adults with cholera-like diarrhea. (From [36])

Table 5.2 Seven point plan for diarrhea control

Package	Treatment and prevention
Treatment package	Fluid replacement
	Zinc treatment
Prevention package	Rotavirus and measles vaccination
	Promotion of early and exclusive breast-feeding and vitamin A supplementation
	Promotion of handwashing with soap
	Improved water supply quantity and quality, including treatment and safe storage of household water
	Community-wide sanitation promotion

United Nations Children's Fund/World Health Organization, 2009

fest cognitive disability, but that there may be a genetic predisposition to this adverse outcome [32]. These studies need to be replicated more widely, and the relative contribution of predisposition vs. insult needs to be elucidated. Beyond genetic predisposition, it will be important to understand the roles of intercurrent infections and the intestinal microbiome in conferring susceptibility to enteric-related morbidity and mortality.

A great deal of research remains devoted to improving oral rehydration. Oral rehydration salts (ORS) with low osmolarity [33], complex starch [34], amino acids [35], and other additives may ultimately provide a material improvement in our ability to avoid adverse outcomes in children who can receive these interventions. Ramakrishna et al. [36] conducted a prospective randomized clinical trial at a tertiary referral hospital in southern India to compare the effects of low osmolarity ORS incorporating amylase-resistant starch instead of glucose (Fig. 5.4). These investigators reported significantly shorter duration of diarrhea in adult males given the maize-based ORT.

Integration of multiple interventions is likely to produce the greatest benefit in terms of lives saved [37]. However, each additional intervention adds complexity to the problems of deployment. Despite decades of international effort, only an estimated 38 % of the world's poorest children receive ORS when they develop diarrhea [38]. The causes for this are many, and

include lack of delivery infrastructure, lack of resources for purchase and dissemination, and focus on other public health priorities. It is likely that developing countries will be able to leverage ORS dissemination programs to introduce other interventions against enteric diseases, providing programmatic synergy.

Fischer-Walker et al. [37] have recently applied a Lives Saved Tool (LiST) analysis to estimate the impact of various interventions in the management of diarrhea. The authors analysed the impact of implementing the following interventions in 68 high child mortality countries: ORS, zinc, antibiotics for dysentery, rotavirus vaccine, vitamin A supplementation and breastfeeding promotion, in addition to basic water, sanitation and hygiene. They found that diarrhea mortality would be reduced by 78 % and 92 %, respectively under an "ambitious" (feasible improvement in coverage of all interventions) and "universal" (assumes near 100 % coverage of all interventions) scale-up impact scenarios. With universal coverage, nearly 5 million diarrheal deaths could be averted during the 5-year scale-up period for an additional cost of US\$ 12.5 billion invested across 68 priority countries for individual-level prevention and treatment interventions, and an additional US\$ 84.8 billion would be required for the addition of all water and sanitation interventions.

Following similar analyses, UNICEF has developed a comprehensive seven-point plan for diarrhea control (Table 5.2). Given the recognition that the burden of enteric disease extends far beyond death from dehydration, success in

the UNICEF plan may realize dramatic improvements in child health.

Perhaps the final frontier in enteric pathogenesis research will be understanding the true cost of exposure to enteric pathogens. Studies in Guinea-Bissau [39] have documented frequent exposure of infants and children to enteric pathogens, with only a minority of the exposures resulting in frank diarrhea. But this observation compels us to ask the key question: Is asymptomatic exposure truly benign? It is well-established that children in developing countries exhibit increased intestinal permeability when compared to their counterparts in industrialized nations [40]. Is this the result of repeated subclinical exposure to pathogens? If so, what is the true cost of this malady?

Many important questions remain in the fight against enteric disease among the world's poorest children. But perhaps for the first time, there exists a confluence of technology that will to bring to bear effective weapons. This effort will require continued application of energy and resources to benefit the majority of the world's children.

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Building Confidence in Vaccines

6

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Abstract

Despite significant efforts by governments, organizations and individuals to maintain public trust in vaccines, concerns persist and threaten to undermine the effectiveness of immunization programs. Vaccine advocates have traditionally focused on education based on evidence to address vaccine concerns and hesitancy. However, being informed of the facts about immunization does not always translate into support for immunization. While many are persuaded by scientific evidence, others are more influenced by cognitive shortcuts, beliefs, societal pressure and the media, with the latter group more likely to hesitate over immunization.

Understanding evidence from the behaviour sciences opens new doors to better support individual decision-making about immunization. Drawing on heuristics, this overview explores how individuals find, process and utilize vaccine information and the role health care professionals and society can play in vaccine decision-making.

Traditional, evidence-based approaches aimed at staunching the erosion of public confidence in vaccines are proving inadequate and expensive. Enhancing public confidence in vaccines will be complex, necessitating a much wider range of strategies than currently used. Success will require a shift in how the public, health care professionals and media are informed and educated about vaccine benefits, risks and safety; considerable introspection and change in current academic and vaccine decision-making practices; development of proactive strategies to broadly address current and potential future concerns, as well as targeted interventions such as programs to address pain with immunization. This overview outlines ten such opportunities for change to improve vaccine confidence.

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Table 6.1 Influenza vaccination coverage among sample of 1931 health-care personnel in US in 2010–2011 by selected characteristics. (Adapted from [6])

Work setting	Vaccinated (%)	Occupation	Vaccinated (%)	Age	Vaccinated (%)
Hospital	71	Physician or dentist	84	18–29	56
Long-term care	64	Nurse	70	30–44	58
Home health	54	Allied health professional	64	45–59	69
Other	47	Assistant/aide	56	≥60	74

6.1 Introduction

Immunization is heralded as one of the most important public health achievements of the last century and without question, vaccines have saved millions of lives and increased life expectancy. However, despite robust scientific evidence showing vaccines to be effective and safe, public doubts persist as they have since the first vaccine efforts by Jenner over two centuries ago. Other factors beyond the availability of evidence are clearly at play [1], undermining immunization programs and the potential for control of vaccine-preventable diseases. This chapter expands on a previous article that outlined several factors undermining confidence in vaccines and that suggested several strategies for how vaccination advocates might increase support and uptake of vaccines [2].

Key topics explored include perception of vaccine risk, the influence of heuristics and beliefs, the impact of the disease control cycle on vaccine uptake, and a discussion of several other factors that influence vaccine confidence. Current strategies to address vaccine concerns are examined followed by an exploration of several different and new opportunities for staunching the erosion of public confidence in vaccines.

6.2 The Vaccine Confidence Deficit

Many factors are involved in the decision to immunize. If the public was fully supportive of vaccines, immunization rates would be well over 95 %. Such is not the case. Lack of confidence or doubt in the safety and effectiveness of vaccines often results in the decision not to immunize. Unfortunately, there are no half measures with

immunization, a person is either immunized or not. Not immunizing, even if this occurs in only a small segment of the population, can undermine the control of vaccine preventable diseases. Public trust in vaccines and immunization programs has to be built; it does not occur by chance and it is not a simple task.

Vaccine confidence is not just a problem for the general public. Even some health care providers remain unconvinced that vaccines are safe, effective, and necessary for health. Although trained to rely on scientific evidence for their decision-making, some also succumb to other influences and question the value of immunization [3, 4]. The persistent annual problem of only moderate uptake of yearly influenza vaccine among health care providers well illustrates this conundrum [5, 6]. Even the threat of an influenza pandemic in 2009 did not drive voluntary compliance rates for the H1N1 vaccine high enough among American health care providers to provide adequate workplace protection [5]. Only health care workplaces that mandated influenza vaccine tended to reach the recommended 90 % target for coverage among their healthcare providers [5]. As shown in Table 6.1, modified from the United States' Centers for Disease Control and Prevention report on influenza vaccination coverage among healthcare personnel for the 2010 influenza season [6], the overall influenza vaccine uptake among the 1931 health care providers sampled was 63.5 % but the rates varied widely by workplace setting, health care worker type, and age.

Vaccine uptake by the public remains suboptimal in many industrialized countries in spite of a wealth of educational materials and a plethora of campaigns and government-funded immunization programs that removed financial barriers to vaccines. With all of this support, one would have

anticipated high uptake of vaccines supplied by governments. Not so. For example, despite easy access to professionally developed evidence-based educational materials on the risks of cervical cancer and the benefits and safety of the human papilloma virus (HPV) vaccine, a publicly-funded school-based HPV vaccine program for girls in British Columbia, Canada, reported only a 65 % uptake in 2008–2009 [7].

Unfortunately, to be truly effective in reducing the burden of circulating vaccine-preventable infections, high immunization rates are required across entire populations. For example, in the case of measles, >95 % of the population must be immune [8]. Outbreaks can occur if the virus is introduced by a visitor into populations <95 % immune, as has recently occurred in parts of Canada [9]. Garnering a behavior compliance rate of this magnitude across entire populations for different vaccine-preventable infections is a daunting task. Few, if any, public health measures require such a high rate of compliance for success. Nurturing confidence in vaccines is thus critical to achieve this goal [10]. Outbreaks in areas where vaccines are easily available but uptake is suboptimal have repeatedly shown just how crucial these high immunization rates are and not just for measles. The pertussis outbreaks in California in 2010–2011, again, illustrate the problem created by emerging pockets of low vaccine uptake [11].

Evidence and fact-based strategies in support of immunization have been used for decades with some success as most parents choose to have their children immunized and many health care providers receive the annual influenza vaccine. But some remain unconvinced and hesitate to immunize despite the evidence, professional recommendations, and easy access to vaccines. To achieve the high vaccine uptake rates required to control diseases such as measles, the unconvinced and vaccine-hesitant minority must also become convinced. The current vaccine promotion strategies are not sufficient to achieve the required vaccine uptake rates.

Among commonly cited reasons for hesitation to immunize are concerns about vaccine safety and the perception that immunization risks out-

weigh the danger of vaccine-targeted diseases [12, 13]. A better understanding of how vaccine-related risks are perceived is crucial for the development of future programs for success. The expansion of vaccinology from health sciences thinking to include that of the behaviour sciences enhances our understanding of why and how vaccine concerns arise despite the obvious benefits of immunization.

6.2.1 Vaccine Perception of Risk: the Role of Heuristics

Many people take pride in making major decisions believing these are based upon conscious and methodical deliberations of available information and evidence. Behaviour scientists, however, have long known that humans are hardwired to deal with threatening situations with reflexive reactions called heuristics (i.e., the use of simple cognitive shortcuts to rapidly solve complex problems) [14, 15]. These automatic mental associations, already biased by previously accessed information and experience, subconsciously influence choices including decisions about health [16]. Whether a parent decides to immunize his or her child or a health care provider opts for annual influenza immunization, automatic associations unconsciously influence the decision-making process. While at a conscious level we may think we are deliberating options and feel undecided, on an unconscious level the decision has already been made. Table 6.2 provides some examples of heuristics and the role heuristics play in vaccine decision-making [2, 14, 16].

One way to think of heuristics is to liken the amalgamation of these reflexive mental associations to a selective brain filter (Fig. 6.1). Beliefs and emotions are on the inside and facts on the outside. While evidence that bolsters preconceived convictions easily passes through the filter, facts contrary to previously held beliefs are met with resistance. Building on the analogy, evidence that can be reshaped in accordance with already held beliefs eventually enters. However, facts that remain contrary to convictions are rejected and may, in fact, serve to strengthen

Table 6.2 Heuristics and Vaccines. (Adapted from [2, 14, 16])

| Heuristic	Simplified definition	Resulting behaviour or belief
Anchoring	Start from a known value or belief i.e. anchor	Healthcare worker sees a serious adverse event following immunization (AEFI) e.g. anaphylaxis following HPV vaccine and now believes AEFI following HPV vaccine to be more common than it is
	Judge probability of a future event by what occurred in past	
Availability	Easily imagined or recalled and therefore judged as frequent or likely to occur	Serious vaccine preventable diseases not seen or recalled, but multiple sclerosis following HBV vaccine misinformation recently heard or seen in the media is believed as correct and common—hence fear of HBV vaccine (France)
Co-incidence dragon	After this therefore because of this	All adverse events that follow immunization must be due to the vaccine. The possibility of coincidence is ignored e.g. autism following MMR vaccine
Compression	Over estimate rare occurrence, under-estimate frequent. Misunderstanding of sample size	Incidence of Guillain-Barré syndrome following influenza immunization perceived by health care provider as more common and serious than influenza complications
Free Loading	Herd immunity will provide protection, no need to immunize myself or my child	Parent believe it is safe to not immunize their child because (1) all other children are immunized (2) the disease is gone. No need for their child to take on "risk" of vaccine
Compression	Over estimate rare occurrence, under-estimate frequent. Misunderstanding of sample size	Incidence of Guillain-Barré syndrome following influenza immunization perceived by health care provider as more common and serious than influenza complications
Omission Bias	Taking action is seen as more harmful than inaction, therefore do nothing	Not immunizing is seen as safer than immunizing. More health care providers took seasonal vaccine than the H1N1 vaccine in 2009–2010 i.e. not to take "new" H1N1 vaccine safer than taking it
Over confidence	Faith in own judgments. Believe they know their own health risks. Think nothing bad could happen to them	Have never had serious influenza and therefore don't need to be immunized. "I am healthy, wash my hands, no need for vaccine"
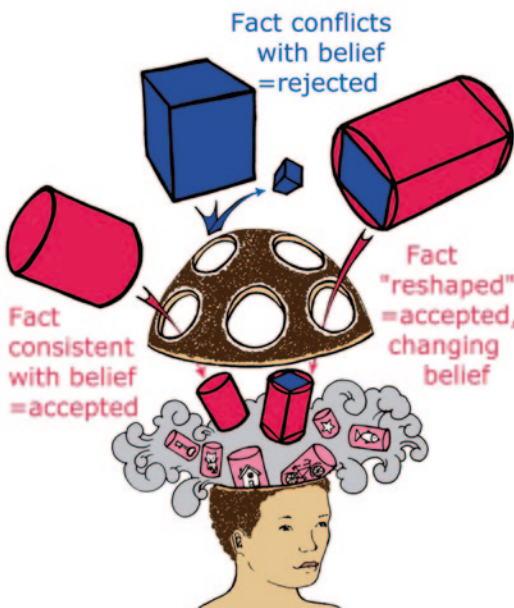
Fig. 6.1 An illustration of heuristics at work filtering fact information. Original art work Kyla Francis with permission

preconceived contrary beliefs, thereby increasing the selectivity of the heuristic-driven filter. While the opinions derived from this process may feel rational, it is really beliefs that are driving the process and consequently biasing conclusions.

Take for example the impact of the *free loading* heuristic on decisions about vaccines (Table 6.2). *Free loading* refers to the belief that other people will assume the risk, so one does not have to put oneself at risk. A parent may choose not to have his/her child immunized because of the belief that sufficient numbers of other children in the community are immunized, thereby providing indirect protection for his/her child through herd immunity. Unfortunately, as has been shown in the pertussis outbreak in California [11], like-minded parents who do not immunize their children often cluster together so the immunization rate in their local under-immunized community is not sufficient for protection through herd

immunity. *Free loading* does not offer adequate protection here. Non-immunized children can develop the vaccine-preventable disease when the disease is introduced into the community and even some of those who are immunized will succumb if they did not respond well to the vaccine [9]. They too were not able to benefit from herd immunity because the overall rate of immunization was not high enough in the locale.

Omission bias, another heuristic, notes that actions are more harmful than inactions. In this case, the risks of getting immunized—an active process—is perceived as carrying more risk than the vaccine-preventable disease—something obtained passively. This perception is incorrect. The H1N1 influenza vaccine saga illustrates this. In 2009, in the face of the H1N1 influenza pandemic, more health care providers were willing to get seasonal influenza vaccine than H1N1 vaccine [5] because the latter was new, untested and hence perceived to present a higher risk. Being immunized for H1N1 was viewed as more harmful than the consequences of not being immunized.

The *over confidence* heuristic refers to placing great faith in one's own judgments. For example, believing that being healthy, eating well, exercising often and carrying out good hand washing will protect oneself from vaccine-preventable diseases like influenza, thereby negating the need for annual immunization. Like the other heuristics in Table 6.2, this and the other automatic thought processes contribute to vaccine hesitancy and suboptimal immunization rates, leaving both the individual and populations at large susceptible to vaccine-preventable diseases.

Politics provides a strong exemplar of the great power of heuristics [17]. In an experiment, an ideology group was given mock news articles that contained either a misleading claim from a politician alone, or a misleading claim followed by a correction. The experiment revealed that subsequent corrections usually did not change the original misperceptions. The subjects remained convinced that the misinformation was correct because the misinformation that supported their beliefs went through their brain filter but the corrections did not. There are many instances where working to correct misinformation have instead further reinforced erroneous beliefs. For example, some people continue to believe that US President Obama was not born in the US, in spite of a verified certificate showing his Hawaiian birthplace. They prefer to believe the misinformation and see the certificate as a cover up and government conspiracy (i.e., their belief has been reinforced by their misinterpretation of the evidence). Heuristics are powerful. The brain filter rejects facts that do not fit the belief. Beliefs begin early, are shaped through maturation, and are sustained through life. In order to avoid selective impermeability to positive vaccine practices, pro-immunization beliefs must be actively shaped and the process started early to maximize better outcomes.

6.2.1.1 The Internet, Heuristics and Perception of Vaccine Risks

Internet search technology also reinforces heuristics. Previous Internet inquiries shape future searches, since Internet search engines are preset to recognize patterns. Google[TM], currently the most utilized Internet search engine, personalizes search results based on previous browsing habits [18]. Combine this with the uneven quality of the health advice that parents can find through Google[TM] [19] and the problem for vaccines becomes clear. For example, one search for vaccine information that leads to an anti-vaccine site triggers the search engine to return to these websites in the next search for vaccine information. This confirms automatic associations in the user's mind between immunization and immunization concerns as highlighted on these anti-vaccine websites. Given that 70–80 % of households in industrialized countries have access to electronically organized and personalized information on the Internet, the Internet has become a major factor influencing human behaviour and decision-making.

The powerful impact of viewing of anti-vaccine websites and heuristics is shown in a German study where even 5–10 min spent viewing an anti-vaccine website had a significant negative impact on vaccine perceptions and decisions to immunize [20]. Another key observation from

this study was that alarming anecdotal cases and testimonials viewed on anti-vaccine websites affected decisions. They easily passed through the brain filter to reinforce negative vaccine beliefs, while fact-based information on the pro-vaccine sites had minimal impact and was repelled by the brain filter.

The arguments against immunization posted on anti-vaccine web sites are also influenced by heuristics, exemplifying serious lapses in reasoning and logic [21]. For example, the natural human desire to find order and predictability in random events can result in the false assumption that events related only by time (temporally) must also be related causally. An example of this *co-incident dragon* heuristic (Table 6.2) is the assumed association between vaccines and autism. Given that autistic features emerge around the time of the immunization at 18 months to two years, causality between these two events has been erroneously assumed. Unfortunately, correcting for false assumptions is not easy. Despite the retraction of the controversial paper by Wakefield that purported a link between the measles, mumps and rubella (MMR) vaccine and autism, that was shown to be both fraudulent and in parts unethical [22, 23], and that Wakefield's medical license to practice medicine in the United Kingdom has been revoked, there are parents who remain convinced of the link between autism and the MMR vaccine [24]. They believe the attempts to discredit Wakefield are a conspiracy (see anti-vaccine website: http://www.naturalnews.com/028101_The_Lancet_Dr_Wake-field.html).

Reshaping of ambiguous and/or poor quality data to fit preconceived hypotheses is another source of erroneous reasoning on anti-vaccine websites [21]. The hypothesis that vaccine-preventable diseases are no longer a threat because of better hygiene and nutrition rather than immunization illustrates this point [21] as does the false assumption that all spontaneous reports of adverse events following immunization must be secondary to vaccines [21]. While many adverse events following immunization are reported to programs such as Vaccine Adverse Event Report-

ing System (VAERS) in the United States [25], many such events are subsequently proven to be unrelated to immunization [26]. However, the association has already been made in minds of many (i.e. the *co-incidence dragon* heuristic). Furthermore, the lack of formal feedback to the health care provider or parent who reported the adverse event, following formal causality assessment to determine if there is a relationship, does nothing to correct any misperceptions. Nor are these individual causality assessment outcomes readily accessible or made easily understandable for the public who have concerns about a specific event. There are no national or international programs that systematically provide a rapid summary of the formal causality assessments to help rectify public misperceptions about specific serious adverse events following immunization. Also, the causality assessment reports provided to vaccine program authorities are often written in very technical language. Even if the public had access to these documents they might not be easily understood.

The public does, however, have Internet access to academic publications and reports by august bodies on vaccine adverse events. Unfortunately, these documents also may not be well understood and conclusions may be lifted out of context and misinterpreted, further feeding negative vaccine beliefs. Public reaction to the August 2011 Institute of Medicine report "Adverse Effects of Vaccines" [26] illustrates this point. This report was written with an academic lens and thus assumes an understanding of medical and scientific terms plus a background in vaccinology. This report provides a rigorous review of the evidence for and against causality from a long list of serious vaccine adverse events following immunization that will be useful for health care providers and vaccine researchers. The lay public, however, may easily misinterpret the findings, as has been seen on Internet blogs following the release of the report. Some have taken parts of the well publicized report to support pre-existing anti-vaccine beliefs, overlooking reference to the very rare serious adverse events and the severity of vaccine-preventable diseases.

Thus even robust immunization data may be intentionally and sometimes even unintentionally misconstrued by anti-vaccine advocates, further undermining trust in vaccines. Some anti-vaccine websites fuel conspiracy theories (see above), suggesting that governments, pharmaceutical companies and even health care experts purposefully suppress evidence that immunizations are dangerous [21]. Heuristics mean that once these beliefs are set, shifting them with facts alone becomes very difficult and almost impossible. Anti-vaccine tales of horror (*availability* heuristic, see Table 6.2) are recalled instead of the evidence that vaccines are safe, effective and important for health.

6.2.2   Other Factors Contributing to the Vaccine Confidence Deficit

Other factors known to influence immunization acceptance include vaccination experience, the disease cycle stage, population-related factors, and the role of experts.

Given heuristics, it not surprising that older vaccines tend to be more trusted than newer ones; that is, the acceptability and safety of older vaccines has already been established, reinforced and is easily recalled (*anchoring* and *availability* heuristics—Table 6.2). In the case of new immunizations, trust must be earned and may not necessarily be transferred from previous vaccines. The earlier example of parents being hesitant about the human papilloma virus vaccine in British Columbia [7], noted above, with only a 65 % uptake rate, contrasts sharply with the high uptake rates for the older well established immunizations for children such as vaccines for measles, mumps, rubella and hepatitis B in the same population. While the *omission bias* heuristic may markedly sway their choices, other factors such as how others in the community are responding may also influence decisions. If parents know that many are refusing then more will.

Vaccine acceptance is also influenced by the disease cycle. A vaccine that prevents a prevalent disease with serious consequences that cannot otherwise be mitigated is highly valued [25]. However, when the disease disappears due to high immunization rates, the vaccine may no longer be seen as important and concerns about vaccine safety may overtake fear of the disease. The automatic assumption to immunize, in order to decrease the threat of disease, is eroded by increasing uncertainty about the value and safety of immunization. Less disease risk leads to more concern about vaccine safety (*anchoring* heuristic, see Table 6.2).

Automatic associations that influence vaccine benefit-to-risk assessments also vary among and within populations, by country and culture, and according to the background experience, environment and knowledge of the decision-maker (e.g., the variation within health-care providers for influenza vaccine as shown in Table 6.1). Context is key.

The role of experts is another factor that can influence immunization decisions. Trust in health care workers is critical to translating advice into action [10], confirming the importance of providers' knowledge of and commitment to immunization. Nurses in particular, because they administer the majority of immunizations, play a significant role as vaccine advisors. The understanding and beliefs held by nurses about the necessity, safety and effectiveness of vaccines not only affects their own immunization behaviour, but also the vaccine practices of others [3, 27]. When health care providers doubt the merit of immunization so do the patients they advise, leading to a negative impact on immunization programs.

In summary, vaccine confidence is indeed complicated and complex with many factors playing into the assessment by the public and health care providers of vaccine benefit, vaccine risks and disease risks. Thus it is not surprising that a 'one size fits all' evidence centered approach to vaccine education and promotion is insufficient to foster and maintain trust. Rather, an immunization program that is more comprehensive, adaptable and responsive is much needed.

6.3 Current Evidence-based Strategies to Deal with Vaccine Concerns

The public health community is well aware that more effort is needed to better address current vaccine safety concerns overall and to meet the needs of different subgroups [11]. While many information materials have been developed for parents these have often been very detailed and complex, many reading more like vaccine package inserts. Hence, a variety of more targeted vaccine information and training materials have been developed by governments and professional groups to better meet the needs of the general public for clear information on risks and benefits. One good example is the quick reference immunization communication tool designed by the Centre for Disease Control in British Columbia, Canada. Written in plain language, this guide provides immunizers with evidence to easily answer common parental vaccine questions and to help explain complex topics [28].

While using evidence and clear language to better answer vaccine-related queries is an important step forward, it does not address the complex behavioral factors noted above. Evidence may or may not get through the heuristic brain filter (Fig. 6.1). Easily understandable well packaged evidence is more likely to pass through and influence decisions but many other factors may prevent even this from happening, as noted above.

New vaccine concerns and questions have arisen for decades and will continue to arise, which means that ever more research will be needed to find evidence that proves or disproves each new allegation. This leaves many vaccine advocates frustrated and puts immunization experts continually on the defensive as they try to respond to these ever shifting concerns while still trying to retain public confidence. Public concerns deserve to be addressed. However, to tackle these unsubstantiated worries one by one through rigorous scientific research requires a substantial commitment of time, money and resources that might be better utilized address-

Table 6.3 Ten novel approaches to consider for enhancing vaccine confidence

1. Exploiting heuristics to benefit vaccines in communication and social media
2. Development of broad awareness of rigorous vaccine safety system
3. Ensure clarity of language in all vaccine communications: public and academic
4. Vaccine science: appropriateness, clarity and quality
5. More open transparent decision making for vaccine approval, programs and policy
6. Employ Strategies to Reduce Vaccine-Related Pain
7. Enhance vaccinology education for health care providers, especially physicians and nurses
8. Proactively educate children on vaccine necessity, benefits and safety
9. Media vaccinology education
10. Facilitate changes in vaccine decision making behaviour with a multi pronged approach

ing other serious health-related issues. Even if evidence-based answers could be provided for all such questions in a very timely fashion (which is almost impossible as it often takes several years to study the question and find the evidence that refutes or proves a new allegation), public mistrust in vaccines would likely persist. Evidence does not necessarily translate into trust. As noted above, negative vaccine anecdotes are much more likely to be recalled than evidence. Hence, a proactive rather than reactive strategy and one that embraces the behavioral understanding of vaccine confidence is required to nurture and foster public support of immunization programs.

6.4 Novel Approaches to Enhance Vaccine Confidence

Given that current strategies have not garnered the confidence in vaccines that is needed and based upon a better understanding of factors that influence vaccine decisions, a number of novel approaches are proposed for consideration in Table 6.3. None of these are mutually exclusive or 'golden arrows' that will nail the target. Some

may garner early wins such as pain control, while others like child education will take much time before they bear fruit. All are likely to interact synergistically.

6.4.1   Exploiting Heuristics for Communications and Social Media

Given that everyone uses heuristics to make decisions, albeit not at a conscious level, vaccine advocates need to understand and exploit heuristics to garner public support for immunization. Two potential heuristics to exploit are *anchoring* and *availability* (Table 6.2). *Anchoring* involves judging the probability of a future event based on what has already occurred, while *availability* involves judging an event as frequent or likely to occur when the event can easily be imagined or recalled. Informing the public of outbreaks of vaccine-preventable diseases in graphic detail can evoke "anchoring" and "availability" heuristics. The clustering of polio cases in Tajikistan in 2010 serves as a useful example. The large outbreak proved that polio can re-emerge, even in regions previously certified as polio-free, if vaccination rates fall below 90 % and asymptomatic cases are imported [29]. In the absence of a disease cure, polio paralysis can be devastating, resulting in death and disability. The image of a child on crutches is heart wrenching to see and readily recalled (*availability*). Hence, describing these unfortunate cases and showing the extent of the outbreak, explaining how the outbreak occurred in a designated polio-free region due to declining immunization rates and emphasizing the potential for similar outbreaks in other certified polio-free regions if immunization rates fall, can make polio real for parents (i.e., easy to recall as it was for parents in the 1950's and 60's because they probably knew someone who had polio). This helps cultivate the belief that vaccines are necessary and important for health. Similarly, the painful complications from the mumps outbreaks in young adults in Canada and the United States, where mumps had previously been controlled [30], provide another excellent
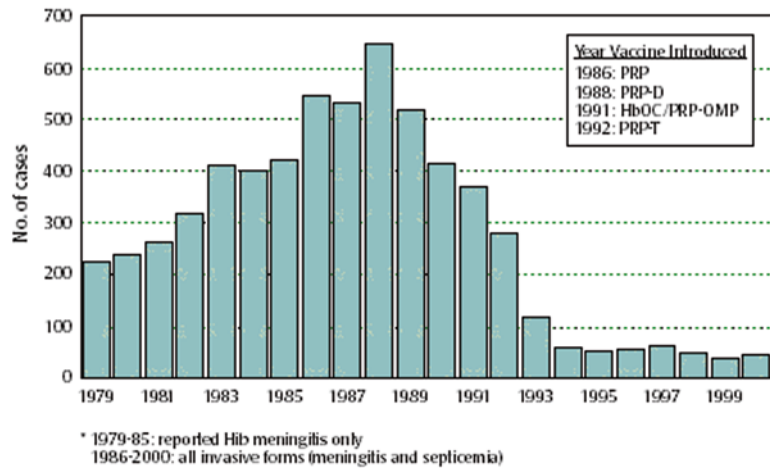
example. A description of the mumps orchitis pain as like having two basketballs on fire provides vivid unforgettable imagery (www.gov. ns.ca/hpp/images/testicular.jpg).

Raising public awareness, especially among parents, of these outbreaks of vaccine preventable diseases and reminding them of the consequences, reinforces *anchoring* and *availability* heuristics. This further nurtures the belief that vaccines are important for health. Parents are then more likely to recall these examples of vaccine-preventable morbidity and mortality when their child is due to be immunized. To craft these messages well takes skills not often present among public health professionals but more commonly found in advertising and marketing experts. Vaccinologists need to develop collaborations with a much broader range of experts than those in health care.

Vaccine advocates themselves should also be educated about historical rates of vaccine-preventable diseases, both locally and globally, and how these rates have waxed and waned with the success and failure of immunization programs [31–33]. Figure 6.2 demonstrates the impact of vaccine on *Haemophilus influenzae b* disease in Canada [31, 32]. Regularly sharing this type of information with the public supports the importance of immunization—again reinforcing *anchoring* and *availability* heuristics for a positive belief in vaccines. It is also worth highlighting where disease resurgences have occurred because vaccination rates dropped as this helps to further refute misinformation that these diseases disappeared due to factors other than immunization [33]. Outbreaks contradict this—vaccines are needed and are powerful. Not being immunized can be tragic. That is the belief that needs to get *anchored*.

In addition, vaccine advocates need to be well aware of the background rates of serious illnesses with unknown and little understood etiologies, such as Guillain Barré Syndrome and multiple sclerosis [34]. As new vaccines are introduced, causality may be inappropriately attributed. Information on background rates can help immunizers and their patients address the flawed reasoning of the *co-incidence dragon* heuristic (i.e., "after it therefore because of it"), as well as the *com-*

Fig. 6.2 *Haemophilus influenzae* type b (Hib) Disease reported cases 1979–2003 in Canada. (Adapted from [33])

* 1979-85: reported Hib meningitis only
1986-2000: all invasive forms (meningitis and septicemia)

pression heuristic (i.e., believing that rare little-understood events are more common than serious disease-related adverse events) (Table 6.2). Many adverse events following immunization, when examined in a formal causality process, are not due to the vaccine but are just co-incidence [35]. As noted above, without transparency about the causality assessment process and a solid feedback loop to those who reported the adverse event following immunization on the findings of the causality assessment, mistrust in vaccine safety will persist among health care workers and the public alike as the *co-incidence dragon* reinforces the belief and is left unchallenged. Communication about the outcomes of causality assessments needs to be well done and well targeted to reach those most involved. The *co-incidence dragon* can be slain if there is only a temporal but not a causal association.

Anti-vaccine advocates well recognize the power of social media, very effectively utilizing the Internet-enabled technologies to garner support for their beliefs. While pro-vaccine websites tend to stick to facts, anti-vaccine Internet sites adopt alternative techniques, including the use of frightening anecdotal stories that easily slide through the brain filter to enhance anti-vaccine beliefs. As described above, based on heuristics, people are more likely to be persuaded by stories and associations rather than stark facts when facing complex decisions. Governments and public health organizations must take this into consid-

eration when designing pro-vaccine websites. They need to better utilize strategies that appeal to heuristics, such as storytelling and anecdotes, in addition to providing evidence. Stories about the terrible outcomes resulting from failure to immunize (e.g., death in children not immunized with *Haemophilus influenzae b* vaccines) [33] helps reinforce and *anchor* the importance of immunization. Fortunately, despite anti-vaccine advocates' efforts to generate an impression of widespread social support for the anti-vaccine stance, most parents continue to support childhood immunization. Vaccination advocates must ensure that parents know this so they will become less susceptible to the anti-vaccine pitch that immunization refusal is the norm in their community or region.

While vaccine advocates have long used printed materials and more recently websites, forays into new social media are now increasing, especially among young parents. Social media has become a normative communication tool for millions. Quality research is needed to ascertain how best to exploit new media; that is, what works well for enhancing confidence in vaccines and what does not. Facebook[TM], Twitter[TM], MySpace[TM], Friendster[TM], Linkedin[TM] and YouTube[TM] can be utilized for communication about vaccines but this takes appropriate skills to do well. Clever use of humour, well crafted messages and respect for the audience requires finesse, but the pay off in audience reach can be astounding.

Dedicated resources are needed to better develop the communication tools used by many parents today as well as future tools that will be utilized by upcoming generations. Pamphlets and television advertisements are not enough.

6.5 Develop Broad Awareness of Vaccine Safety System

Recognizing the importance of heuristics in decision-making, parents and health care providers must be continually reminded of the rigorous safety evaluation of all vaccines [36]. Doing so can help bolster trust in immunizations as well as help filter out vaccine safety misinformation. Unfortunately, most industrialized countries have invested little in educating health care providers and the public about the strength and reliability of their vaccine safety systems that are indeed more fulsome and rigorous than the drug safety systems. The first supplement of the National Vaccine Program Office of the US Department of Health and Human Services, outlining vaccine safety throughout the product cycle, was only published in 2011 [37]. Of note, an article in this supplement outlines how the Vaccine Safety Datalink system can be used to quickly check on purported adverse events linked to a vaccine, to determine if there is sufficient evidence to stimulate a more in-depth assessment [38] but there is no information on whether this is then reported back to the health care workers or parents who initiated the reports.

The Cuban government long ago recognized the importance of garnering community support for vaccines. Despite the fact that polio was eradicated from Cuba over 30 years ago, a national annual polio immunization program continues to flourish. Involving thousands of mothers and children, under the auspices of the Cuban Federation of Women, the neighborhood Committees for the Revolution and Public Health, this annual public show of support for immunization reinforces the community's trust in vaccines [36]. The Cuban national vaccine education program, starting with school-aged children and continuing into adulthood, further bolsters national confidence in vac-

cines. Interestingly, Cuba has not suffered from the vaccine safety angst seen elsewhere. Indeed, other countries can learn much from Cuba, where vaccine education is an integral part of citizenship and trust in immunization is the norm.

6.6 Clarity of Language

Academic and overly-scientific language can unintentionally subvert efforts to educate the public about vaccines. While scientific journals and technical academic reports used to be the purview only of those trained to read and interpret them, the widespread availability of these materials via the Internet, as noted above, has created the potential for misuse and misunderstanding, especially by lay readers including the media.

Academic jargon can easily obscure the intended scientific meaning, confusing untrained journalists and parents, and fostering misinterpretation by anti-vaccine advocates [39]. This can lead to anti-vaccine websites using misinformation in support of their beliefs. The Internet provides ready access to a multitude of scientific reports, including those from august bodies such as the Institute of Medicine in the United States and the National Institute for Health and Clinical Excellence in the United Kingdom and to a wide array of open access academic journals. Thus, greater care is now needed to ensure that the conclusions, implications, and executive summaries in academic articles and reports are easy to understand and are not obfuscated by jargon [39].

Unfortunately, the recent trend has been away from clear plain language in many academic articles. Technical reports are also becoming longer and more complex often with hundreds of references, making clarity of language in the executive summary and the final conclusions even more crucial as these will be the most read sections. Few people will read an exhaustive report in its entirety. The vaccine recommendations from the national immunization technical advisory groups (NITAGs) like the United States Advisory Committee on Immunization Practices (ACIP) exemplify this point. Even experienced

users may struggle to find and interpret the major recommendations made by these advisory committees [40]. Ambiguous, complicated language and academic jargon are confusing for many, including many health care providers. Clarity in communications must become the norm whether they be academic articles, technical reports or advisory committee recommendations. As Einstein noted, "*Make everything as simple as possible, but not simpler*".

6.7   Vaccine Science: Appropriateness, Clarity and Quality

Polland and colleagues have also emphasized that great care must be used when research tools such as meta-analyses and evidence-based medicine techniques are applied to vaccines [41, 42]. Evidence-based analyses value outcomes such as infection rates much more than proxy outcomes such as immunogenicity. For rare diseases such as meningococcal infections or rabies, this would mean vaccine studies would require huge populations followed over several years for randomized controlled trials, an expensive and untenable position. Emphasizing such well-used research tools could lead to an undervaluing of vaccines studies compared to other drugs where more classic end points are used. This can lead to the erroneous implication that vaccine science is somehow less rigorous, thus less valuable and less credible. Similarly, assessing the findings from tools like meta-analyses needs to be used with care for vaccines, given their potential for undermining immunization program communications.

Journal editors also need to be reminded of the critical role vaccines play in population health. Not only must they critically assess the language of submitted articles for clarity and ease of understanding but also take great care in assessing the scientific quality of submissions. Poor quality science, when published in esteemed journals, has the potential to undermine public and professional confidence. Because high rates of vaccination are needed to control disease, even a small shift in confidence because of a well publicized academic article, such as occurred with the Wakefield article noted above, can lead to deaths. Much damage had already been done by the time that article was withdrawn years later.

While not suggesting that editors should decline to publish articles that cast doubt on vaccine benefits or highlight risks, they must bear in mind and be cautious of ecological and epidemiological studies that purport to show vaccine associations with diseases of unknown etiologies, such as multiple sclerosis, as the observed association may well be due to chance alone and not be causally related.

6.8   More Open Transparent Decision Making

Many anti-vaccine websites abound with allegations that governments conspire to suppress and even distort negative information about vaccines. These allegations are hard to refute when discussions and decisions about public funding of vaccines and programs, vaccine licensure, and vaccine safety assessments are not done openly. Although there has been some movement towards more open and transparent processes by some NITAGs and drug licensing bodies (i.e., ACIP in the US) [40], other organizations have lagged behind. As well, oft times, conflicts of interest in decision-making remains shielded from public scrutiny. Anti-vaccine websites harp on this flaw. This is not trivial. Previous failures to bring medication safety information to the public's attention in a timely fashion as occurred with Vioxx[TM], has led to an erosion in the public's confidence in drugs and the pharmaceutical industry in general [43]. This experience has only added fuel to the belief of many that vaccine problems are also being covered up. This is heuristics at work, augmenting public doubt in vaccines. Transparency is crucial.

Moving to a model of more robust disclosure will not be easy. NITAGs and drug licensing bodies will require a better understanding of the public's concerns about vaccine decision-making and then an open mind when considering how best to address these transparency concerns. Overly defensive responses to calls for more public scrutiny need to be avoided. Instead, organi-

zations must learn to address public inquiries in a sensitive manner. Commitment to an open and transparent vaccine system can help enhance the public's confidence in vaccines and undermine anti-vaccine conspiracy allegations.

6.9   Strategies to Reduce Vaccine-related Pain

Pain from immunization has been long neglected as a cause for vaccine hesitancy, overshadowed by other vaccine-related concerns. However, pain with immunization is a significant source of distress for children, parents and health care providers—a concern expressed by 44 % of parents in one survey [44]. Fear of needles and anxiety about procedures can negatively impact vaccination and health-seeking behaviors [45]. In 2010, an evidence-based Canadian clinical practice guideline intended to reduce pain related to childhood immunization was published [45]. Designed by an interdisciplinary group of experts in pain and in vaccinology and approved by several professional societies, the guideline has been met with considerable interest by both immunizers and parents in Canada. Reducing pain with immunization is a step in the right direction to decrease vaccine hesitancy and increase vaccine uptake. Ignoring this for so long has been folly. However, the effectiveness of these approaches across populations and settings still requires thoughtful evaluation. Getting pain control right is important.

6.10   Vaccine Education for Physicians and Nurses

Formal education in medical and nursing schools has not typically provided sufficient information and training to prepare physicians and nurses to answer the vaccine-related questions from patients that often arise in their practices [46]. Low immunization uptake rates for annual influenza vaccine among health care providers, as noted above, demonstrates that physicians and nurses have not all been convinced during their training and while out in practice that immuniza-

tion is necessary to reduce the burden of diseases such as influenza. Education must be improved. In addition to enhancement of vaccine education in medical and nursing school, standardized competencies for those who immunize, such as the Immunization Competencies published by the Public Health Agency of Canada [47], could further reinforce the importance for health care providers of immunizing themselves and their patients. Given the important role health care providers play in patient vaccine decision making [12, 27], the training of these professionals needs urgent attention by curriculum planners and much pressure from vaccine programs and governments. Having a health care work force undereducated about vaccines critically undermines vaccine program success.

6.11   Vaccine Education for Children

Building confidence in vaccines should start early. A basic explanation of vaccines, including how they work and how vaccine safety is monitored, could be part of the health curriculum for elementary students and then reinforced in secondary school. Immunization is such an important public health measure that its significance could be incorporated into history and science classes as well. This would begin the process of building trust and shaping positive vaccine beliefs at an early age, a powerful technique for shaping adult decision making. There are a variety of teaching approaches that have been shown to make vaccine information meaningful to young students including drawings, word puzzles and stories of real people who have experienced vaccine-preventable diseases [48]. Figure 6.3 shows an example of a student's poster drawn after participating in such a program.

6.12   Vaccine Education for the Media

While health care providers play a major role in parental decisions to immunize their children [12, 27], what appears in the media, both tradi-

Fig. 6.3 Immunization: protecting you, your family and your community. Ryan McGee winner 2008 National Immunization Poster Context. Canada. Public Health Agency of Canada. With permission
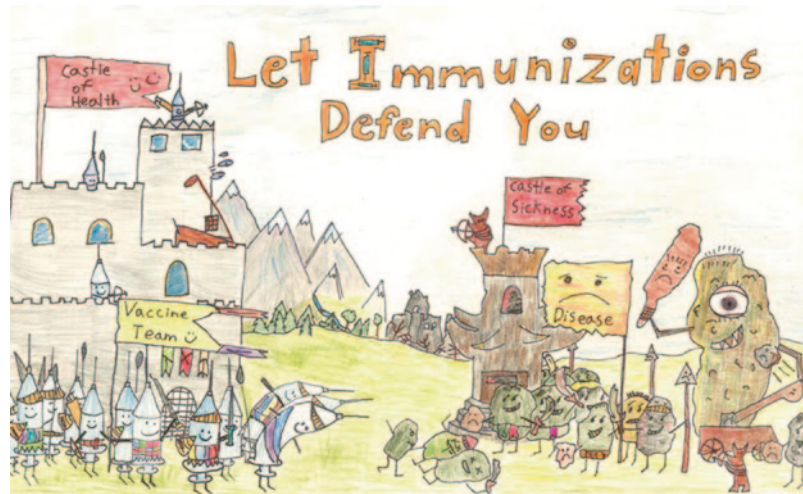
tional print and broadcast media as well as social media, is also influential as noted above [20]. Heuristics are at work here. Media stories that reinforce beliefs are well heard but those that disagree are filtered out. If there are no preconceived beliefs about vaccines, media stories may shape these beliefs, pro or con, depending on the story.

Unfortunately, vaccines are variably portrayed in the formal media. A 2011 report of a 10 year survey of vaccine-related articles in U.S. daily newspapers, with circulations equal to or over 50,000, found that 37 % of the articles suggested that vaccines were unsafe [49]. Over 60 % of all the vaccine articles were written by newspaper or wire service staff, many of whom likely only had a rudimentary background in science and probably no background in vaccine science. Recognizing the importance of journalists in shaping public opinion, many organizations that advocate for vaccines distribute news releases and backgrounders for the media, hoping journalists will simply use their vaccine facts in their articles. However, journalists are trained to question traditional sources and to present 'the other side' of the story which sometimes means the anti-vaccine side. As traditional media outlets experience more and more staff cuts, there are now fewer journalists who focus solely on science or health issues. Even fewer have substantive enough health or science expertise to fully brief themselves on the issues presented. Hence, it is

increasingly important to simplify the knowledge translation of vaccine science and to make the major findings in academic articles crystal clear, not only in the covering news release but also in the document's conclusions as noted above.

Ideally, journalists could attend educational workshops and seminars on vaccines, such as the European Media Workshop organized by the Fondation Mérieux in Annecy, France, April 2009, aptly titled "Unjustified scare or reasonable skepticism" [50]. Similar educational workshops for journalists have been offered at the biannual Canadian Immunization Conferences. While the importance of these education opportunities for media has not been formally assessed, this may be a promising avenue that leads to enhanced confidence in vaccines in the media and, by extension, the public. It is interesting to note that one of the most prominent health reporters in Canada who writes for the largest national daily is well educated about vaccines and is a staunch supporter of vaccines [51].

Given that annual national immunization weeks as well as regional and national immunization conferences are becoming more common throughout the world, journalists could be encouraged to not only report on the vaccine events but also to participate in short courses on vaccine science (online or in person) designed specifically for them. Other tools such as online briefings and backgrounders could also be made

readily available to journalists including explanations of basic vaccinology terms and concepts relevant to the study—all provided in lay language. Expert spokespeople should also use plain language, and be clear about the implications of reports and articles. As outlined above, obfuscation is an open door to misunderstandings.

Working with social media, especially bloggers, presents an enormous challenge as there are so many of them. Blogs should be monitored and those with large followings responded to wherever possible if vaccine misinformation is being promulgated. While this takes time and effort, as politicians have well learned, you ignore bloggers at your peril.

6.13   Facilitate Changes in Behaviour

Most beliefs and behaviours are deep rooted, difficult to change, and well established by adulthood. Modifying beliefs and behaviors is thus, a complex process. There is no one way to build confidence in vaccines among adults that leads to high vaccine uptake. As noted above, sowing pro-vaccine belief seeds in childhood may be key but this will take at least a generation before accruing the benefits. What can be done in the interim? For adults, multiple approaches will be required, including many outlined above. Positive public vaccine beliefs will need to be actively shaped and reinforced.

6.13.1   How can this be Accomplished?

One model to consider is the World Health Organization Communication-for-Behavioural (WHO COMBI) program, a comprehensive, people-centered approach used to facilitate behavioural change at individual, family and societal levels [52]. Although typically applied to diseases in developing countries, the WHO COMBI strategy may be amenable to immunization programs nationally and globally. Drawing on the Cuban example noted above, comprehensive national immunization strategies that effectively influence behaviour can be highly successful. The potential of a COMBI like program for immunization deserves serious exploration.

As well, national vaccine programs need to involve a broader range of disciplines, expanding beyond traditional players with health backgrounds, to include contributions from the behaviour sciences, marketing, and communication. These disciplines would bring a wealth of new expertise and knowledge to the development of immunization programs and the field of vaccinology. Strategies for building vaccine confidence locally can be much stronger if they draw on local capacity to build robust immunization programs. Engaging community members in finding solutions to the vaccine confidence deficit problem will be fundamental work here as the literature has well shown that top down solutions often fail [53].

6.14   Conclusion

Fact-based communications and effective programs have achieved the high rates of immunization needed to control many vaccine-preventable diseases. These strategies need to continue. However, while many people have been convinced by scientific evidence, the vaccine-hesitant minority have not. Given that very high immunization rates are required for disease control across populations, ignoring the vaccine hesitant is not an option. The vaccine hesitant more often rely on beliefs and automatic assumptions and are not influenced by evidence. As stated by Goethe, a German polymath, over 150 years ago, "*Belief is not the beginning but the end of all knowledge*". He continued, "*We are so constituted that we believe the most incredible things; and, once they are engraved upon the memory, woe to him who would endeavour to erase them*".

A proactive, multidisciplinary, multifaceted approach capable of reshaping immunization beliefs is needed to address the vaccine confidence deficit. Devising such a strategy will not be easy. Vaccine advocates need to collaborate with experts in the behaviour and social sciences, in marketing and advertising, as well as members of the community to bring more depth and breadth

to immunization programs. Milestone evaluations will be needed to ensure that the devised programs achieve the stepwise desired goals. Fortunately, there are some successful models to examine and build upon including the Cuban national immunization program and the WHO COMBI strategy for dengue fever control.

Vaccines are a critical public health strategy for population health and well being. The trust, support and confidence of health care providers and the general public in vaccine programs is essential for success. Given the high costs of failure, including health care costs and needless morbidity and mortality from vaccine-preventable diseases, immunization advocates need to aggressively address the vaccine confidence deficit at its roots by actively working to shape vaccine beliefs.

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Chronic Recurrent Multifocal Osteomyelitis

7

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Abstract

Chronic recurrent multifocal osteomyelitis (CRMO) is an autoinflammatory bone disease occurring primarily in children and adolescents. Episodes of systemic inflammation occur due to immune dysregulation without autoantibodies, pathogens or antigen-specific T cells. CRMO is characterised by the insidious onset of pain with swelling and tenderness over the affected bones. Clavicular involvement was the classical description; however, the metaphyses and epiphyses of long bones are frequently affected. Lesions may occur in any bone, including vertebrae. Characteristic imaging includes bone oedema, lytic areas, periosteal reaction and soft tissue reaction. Biopsies from affected areas display polymorphonuclear leucocytes with osteoclasts and necrosis in the early stages. Subsequently, lymphocytes and plasma cells predominate followed by fibrosis and signs of reactive new bone forming around the inflammation. Diagnosis is facilitated by the use of STIR MRI scanning, potentially obviating the need for biopsy and unnecessary long-term antibiotics due to incorrect diagnosis. Treatment options include non-steroidal anti-inflammatory drugs and bisphosphonates. Biologics have been tried in resistant cases with promising initial results. Gene identification has not proved easy although research in this area continues. Early descriptions of the disease suggested a benign course; however, longer-term follow up shows that it can cause significant morbidity and longer term disability.

Although it has always been thought of as very rare, the prevalence is likely to be vastly underestimated due to poor recognition of the disease.

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7.1 Introduction

Chronic recurrent multifocal osteomyelitis (CRMO) is an inflammatory bone disease occurring primarily in children and adolescents. It
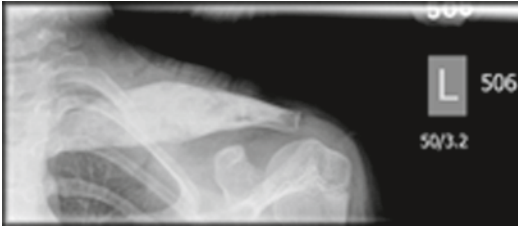
Fig. 7.1 Plain radiograph showing the left clavicle expansion and sclerosis of entire shaft

was first described in 1972 by Giedion as "an unusual form of multifocal bone lesions with subacute and chronic symmetrical osteomyelitis" [1]. Subsequently, Björkstén called it CRMO, describing nine patients with fever, pain and radiological findings suggestive of osteomyelitis and described recurrent pustulosis palmaris and plantaris, which paralleled the exacerbations in bony lesions [2].

There are now around 300 cases of CRMO described in the literature, predominantly as case series. It is difficult to guess the true prevalence as it is likely that as a little-known disease it is underdiagnosed [3]. CRMO is related to Synovitis, Acne, Pustulosis, Hyperostosis, Osteitis (SAPHO) syndrome which may represent a similar disease process in adults [4].

CRMO has recently been classified as an auto-inflammatory disorder (rather than autoimmune). These inflammatory conditions are characterised by episodes of systemic inflammation including serological signs of inflammation occurring in the absence of autoantibodies, pathogens or antigen-specific T cells [5–7]. They appear to be caused by a primary dysfunction of the innate immune system [8].

7.2 Clinical Features

The consistent feature of CRMO is the insidious onset of pain with swelling and tenderness localised over the affected bones. Lesions may occur in any bone, including vertebrae [9–17]. Involvement of the clavicle is classical however, the metaphyses and epiphyses of the femur, tibia or humerus are also frequently affected. Involve-

ment of the skin includes palmoplantar pustulosis, occurring in approximately 20 %, acneiform lesions or psoriasis [2, 15, 18–20]. Inflammatory bowel disease, particularly Crohn's-like lesions have been associated with CRMO and may represent "enteropathic CRMO" [21–25]. There is a female preponderance with twice as many girls affected and a median age of onset of around 10 years [3, 5, 26, 27].

Fever may be a feature during episodes of disease flare, and inflammatory markers such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) level, leucocytes, and fibrinogen are commonly elevated. However, the increases are usually moderate and return to normal during quiescent periods. If these markers are very significantly elevated it favours an alternate cause and this should be investigated further. [5, 10, 16, 27, 28]

7.3 Radiology

Plain x-rays may be normal in the early stages of disease, but later will show the usual features of osteomyelitis, such as osteolysis, sclerosis, and new bone formation, or combinations of these. Different patients have very different radiological findings with some lesions showing periosteal reactions and lytic areas mimicking tumours [29] (Figs. 7.1 and 7.2).

Characteristic imaging findings include bone expansion, medullary lytic areas, periosteal reaction, bone oedema, and inflammatory soft tissue reaction. These features may help distinguish the diagnosis from other diseases [5].

Radionuclide bone scan are still used in some departments to identify silent lesions in other bones (as foci of increased uptake) and to monitor the response to therapy. However, whole body MRI using short tau inversion recovery (STIR) sequencing permits identification of the characteristic features of areas of bone marrow oedema (hyperintensity on STIR images). It is also more sensitive than radiographs or bone scans to identify silent lesions without radiation. In many cases this will help identify multifocal lesions

Fig. 7.2 Bone scan demonstrating increased areas of uptake in the left clavicle and both proximal humeri
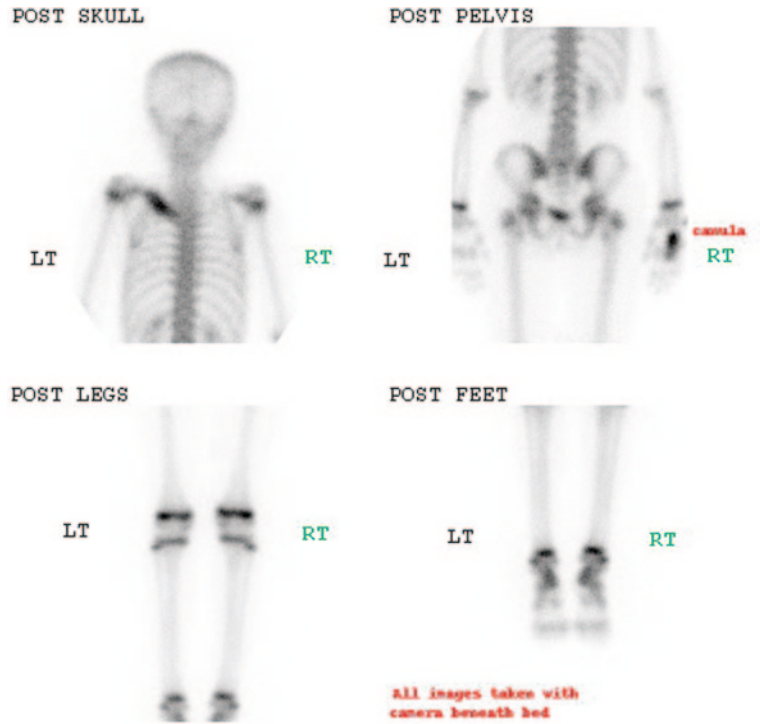
Fig. 7.3 MRI stir coronal image showing high signal in right proximal humerus
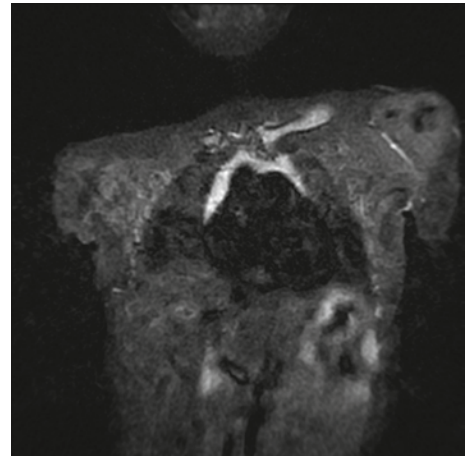
Fig. 7.4 MRI stir coronal image showing high signal in the shaft of left clavicle

where only one has been noticed clinically and make the diagnosis plain [30] (Figs. 3 and 4).

If the clinical presentation, laboratory findings and radiology strongly suggest CRMO then, potentially, the need for a bone biopsy can be avoided [5, 27].

7.4 Diagnosis

Various diagnostic criteria have been suggested [9, 15, 16] but essentially CRMO remains a diagnosis of exclusion, with the association of positive and negative findings. Multifocal bone lesions

are rare in childhood but can have a diverse aetiology including leukaemia, neuroblastoma, Langerhans cell histiocytosis and staphylococcal osteomyelitis.

CRMO is particularly difficult to diagnose when there is only one bony lesion; care must be taken to exclude bacterial osteomyelitis which may only be possible after biopsy, failure to respond to antibiotics and then a good response to non-steroidal anti-inflammatory drugs without antibiotics [15].

7.5　Pathophysiology

Bone biopsies from affected areas show non-specific inflammation. There is a spectrum of changes beginning with an acute inflammatory process with early lesions consisting of polymorphonuclear leucocytes accumulating in the marrow. At this stage there is also osteoclastic bone resorption and necrosis. Subsequently, lymphocytes and plasma cells become more common in the inflammatory infiltrates and some cases display granulomatous foci. Fibrosis occurs in the later stages and osteoblasts are seen with signs of reactive new bone formation around the inflammation which parallels that seen radiologically as osteosclerosis [31, 32]. Due to the wide variation of reparative changes seen, histological examination alone cannot differentiate CRMO from bacterial infection [9, 33–35].

7.6　Management

There is currently no way of identifying those patients that will have spontaneous resolution of symptoms and those who will have persistent disease. In all cases with active disease, the bone pain may be severe, resulting in reduced quality of life, disruption of childhood and potentially permanent bony deformities [36–39]. The aims of treatment are to minimise pain and maintain normal bone growth and function of adjacent joints [40]. There are no published randomised controlled trials of treatment in CRMO.

7.6.1　Nonsteroidal Anti-Inflammatory Drugs

Nonsteroidal anti-inflammatory drugs (NSAIDs) are first-line treatment and may be very effective; producing a clinical and radiological improvement in many patients. One case series found indomethacin provided a dramatic response, which was sustained throughout the four year follow-up [41]. Naproxen has also been used with good effect for both primary lesions and in relapse. [29, 42] In a review compiling published case reports of CRMO, overall 79 % of patients had a good response to a variety of NSAIDs [15].

None of these studies were randomised controlled trials; however, as Geschick points out, if duration of symptoms is compared with the historical controls first described by Björkstén et al. [2], there is a marked difference in duration in favour of NSAIDs [42, 43].

If there is a failure to respond to one NSAID it is worth trying one or two others, as the response is recognised to vary between patients [9, 26–28]. It is postulated that NSAIDs act by inhibition of the cyclooxygenase pathway and thereby inhibiting prostaglandin synthesis [29].

7.6.2　Steroids

For those patients who fail to respond to NSAIDs, steroid therapy with intravenous methylprednisolone, oral prednisolone or hydrocortisone has been used with good effect. However, the resolution of pain may be transient with symptoms recurring once steroids are stopped [9, 29, 44, 45].

7.6.3　Disease Modifying Agents

Methotrexate and azathioprine are generally considered to be ineffective [9].

7.6.4　Pamidronate

Evidence is building for the use of the bisphosphonate pamidronate in CRMO when NSAIDs

have failed to control symptoms. It is generally very effective in reducing pain, sometimes dramatically; with improvement of functional outcomes and evidence of reduction in lesion size as shown by a variety of imaging methods [39, 46–48]. It has also been shown to resolve vertebral hyperintensities (seen on MRI) either partially or completely, with improvement in vertebral modelling and height [14, 49].

Pamidronate was the first bisphosphonate to be used in CRMO as there was already experience of its use in a paediatric population in osteogenesis imperfecta [50]. The same three-day paediatric protocols were used. Neridronate (an aminobisphosphonate) has also been used in CRMO and appears to have similar effects to pamidronate [51].

The mechanism of action remains uncertain. Bisphosphonates are known to inhibit osteoclasts [52] (which are seen in biopsies of lesions), and may reduce lesion expansion by action at this site. Using bisphosphonates as anti-cytokine agents in inflammatory conditions is becoming more widespread [53] with effects on the release tumour necrosis factor-α and interleukin-6 from T-lymphocytes [54–55]; Minor flu-like symptoms and a mild increase in pain for 24 h are common with pamidronate infusion and are found most frequently after the first infusion. Subsequent infusions tend to be better tolerated [56–57]. Although the rapidity of resolution of pain in patients whose pain has been resistant to NSAIDs lends weight to the efficacy of bisphosphonates for treatment of CRMO [46]. Randomised controlled trials would be helpful to explore the efficacy and potential adverse effects of this relatively recently described use of bisphosphonates.

7.6.5 Interferon

There are some case reports of good results using interferon but they have been superseded by the use of biological agents in CRMO resistant to NSAIDs, bisphosphonates or steroids [58, 59].

7.6.6 Biologics

Initial reports are being published on the use of biological agents in CRMO. However, it must be remembered that the patients treated with biologics represent a subset of patients with disease that is resistant to NSAIDs, pamidronate and, in some cases, steroids.

Infliximab is a chimeric monoclonal IgG1k antibody with action against tumour necrosis factor-alpha (TNF-α) and has been used in a handful of patients in varying doses with mostly positive results.

A large (89 patients) retrospective cohort study found two patients had been treated successfully with infliximab [9]. Another child with CRMO followed by Crohn's disease also had a good response to infliximab with resolution of pain and decrease in size of the bony lesion [22]. A further case report using a lower dose of 3 mg/kg at the same intervals led to resolution of pain at the third dose, and continuing for the 2 years of follow-up. In this instance, bone scans were used to demonstrate radiological improvement [60].

With less promising results, a case report of two children receiving Infliximab found that although both patients initially showed improvement of symptoms, one child had to increase treatment to every 4 weeks due to a recurrence of symptoms with azathioprine added before symptoms were fully controlled; the second child stopped infliximab due to a possible fungal skin infection [61]. Using a different dosage, a child with CRMO resistant to all other treatments was treated with infliximab (5 mg/kg) every 4 weeks for 12 months and then at 8 weekly intervals. This resulted in remission of symptoms and allowed the cessation of NSAIDs and steroids [62].

There is one published report of two cases where etanercept (a recombinant, humanised TNF receptor which antagonises soluble TNF) was used in combination with methotrexate and led to clinical remission [63].

The role of TNF in the progression of CRMO is unclear. Whilst the case reports of efficacy of TNF blockade suggest some involvement, the mouse model of CRMO has not implicated TNF as the major factor [64].

Anakinra (recombinant IL-1ra) was used in one patient with CRMO having first demonstrated marked elevation of interleukin 1ra. Using a daily dose of 2 mg/kg, there was a good response initially that persisted for 17 months before further symptoms developed and, after 3 years, necessitated switching therapy to adalimumab (humanized IgG1 anti-TNF-α mAb) [61].

There is more evidence for use of biologics in patients with SAPHO, in whom an increase in TNF-α has been well described [65–70].

7.7 Genetics

There are several reports of family members being affected, including two pairs of monozygotic twins, suggesting a genetic susceptibility [9, 18, 71].

A mouse with a spontaneous mutation on chromosome 18 following an autosomal recessive inheritance was found to have similar abnormalities to human CRMO [72]. Two human disorders of bone have been localised to human chromosome 18q [73, 74] and therefore this seems like a possible area for a susceptibility gene. A significant association of CRMO with a rare allele of marker D18S60 microsatellite polymorphism suggests the existence of a susceptibility gene on chromosome 18 which may contribute to the aetiology of CRMO [71].

A missense mutation in PSTPIP2 in the chronic multifocal osteomyelitis mouse [75] led to this being investigated as a candidate gene; however, a series screening ten patients with CRMO for mutations in the genes PSTPIP1 and PSTPIP2 found no mutations [9].

From studies to date it is not possible to infer a monogenic inheritance pattern.

7.8 Outcomes

The course of CRMO is very variable with symptoms remitting and relapsing. The disease was initially thought to resolve spontaneously, going into remission particularly during puberty. However, as follow-up studies grow in length, it is apparent that many patients continue to have long-term impairments, such as bony deformity, disability and chronic pain [10, 11, 37]. Some patients evolve into an atypical spondyloarthropathy [20].

7.9 Conclusion

It is now 30 years since CRMO was first described but recently our understanding of its nature has changed. Rather than being an auto-immune disorder as first thought, it is now thought to be one of the autoinflammatory disorders, where systemic inflammation occurs due to a dysregulation of the immune system. Although it has always been thought of as very rare, the prevalence is likely to be vastly underestimated due to poor recognition of the disease. Diagnosis may be made easier by the advent of STIR MRI scanning, potentially obviating the need for biopsy and avoiding unnecessary long-term antibiotic treatment due to incorrect diagnosis. Treatment options are increasing with an expanding evidence base for the use of bisphosphonates. It is early days for the use of biologics but initial results appear promising in resistant cases. Gene identification has not proved as easy as first thought when a mouse model was identified, although research in this area continues.

The early descriptions of CRMO suggest a benign course; however, longer-term follow up is showing that the disease can cause significant morbidity and longer term disability.

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Recognition and Treatment of Chlamydial Infections from Birth to Adolescence

8

Toni Darville

Abstract

The "silent epidemic" of *Chlamydia trachomatis* threatens to cause reproductive damage and infertility in many of the 50 million women who acquire it each year. Female reproductive tract infection has more recently been linked to stillbirth and premature delivery. Innate immune cells and mediators appear to be the primary players in pathogenesis, with neutrophils playing a prominent role in disease development. Although adaptive antibody and CD4 T cell responses appear primarily protective, these responses are inefficient. Infections are frequently chronic as a result, and when infection is diagnosed and treated with appropriate antibiotics, repeated infection is the rule. The lack of acute symptoms in many infected individuals contributes to the high prevalence of chlamydial infection. Although chronic sequelae are relatively rare in men, and many women sustain infection without developing pelvic inflammatory disease or chronic sequelae, the extremely high prevalence of chlamydial infection leads to significant morbidity and healthcare costs. A vaccine is urgently needed to prevent infection, but given the difficulties of inducing a CD4 T cell memory response that can home quickly to the genital tract, induction of sterilizing immunity may not be possible. A vaccine that prevents disease by lowering bacterial burden and dampening production of tissue-damaging responses may be possible. Until an efficacious vaccine is developed, screening and treatment programs appear to be the best method of disease prevention.

8.1 Introduction

Chlamydia trachomatis is an obligate, intracellular, nonmotile, Gram-negative bacterium recognized as one of the most common sexually transmitted agents in the world. Chlamydial genital infection primarily affects sexually

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active adolescents and young adults. Large-scale screening programs routinely detect infection rates of 5–10 % in young adults (19–25 years of age) [1, 2], and 10–20 % or greater in sexually active adolescents 15–19 years of age [3]. Most infected persons do not have symptoms, thus they do not seek medical care, and their infections go undetected. Consequently, screening is necessary to identify and treat this infection. The large reservoir of unrecognized infected individuals helps sustain transmission of this organism. Among men, urethritis is the most common illness resulting from *C. trachomatis* infection. Complications (e.g., epididymitis) affect a minority of infected men and rarely result in sequelae. Women bear the brunt of disease due to infection, for if left untreated, infection can ascend from the cervix to infect the uterus and Fallopian tubes to cause pelvic inflammatory disease (PID). Inflammation of the Fallopian tubes can lead to subsequent scar formation, and tubal occlusion. Tubal obstruction can lead to ectopic pregnancy, subfertility and infertility. In addition, an infected pregnant woman can transmit the organism to her newborn at the time of delivery, potentially resulting in neonatal conjunctivitis and/or afebrile pneumonia.

8.2 The Pathogen

Chlamydiae are obligate intracellular parasites that have been classified under the order Chlamydiales with their own family and genus (Chlamydiaceae, *Chlamydia*). Chlamydiae are small in size (0.25–0.8 μm in diameter) compared with typical bacteria such as *Escherichia coli* (1.0 μm) and have small chromosomes ranging from 1.0 to 1.2 megabases in size. They are Gram-negative in architecture and composition, with an outer membrane containing lipopolysaccharide (LPS), which is truncated, and not very endotoxic and a cytoplasmic membrane. While the classic bacterial cell wall component peptidoglycan has not been confirmed by isolation and identification, chlamydiae possess all the genes needed for its synthesis and are susceptible to β-lactam antibiotics [4]. Although chlamydiae contain DNA,

RNA, and ribosomes, during growth and replication these obligate intracellular bacteria parasitize their host epithelial cell for nutrients and are auxotrophic for several amino acids and three of the four nucleoside triphosphates; the demand for host cell adenosine triphosphate (ATP) has led to their designation as an "energy parasite." *Chlamydia trachomatis* encodes an abundant protein called the major outer membrane protein (MOMP or OmpA) that is surface exposed and is the major determinant of serologic classification. Almost all strains of *C. trachomatis* harbor a plasmid, which confers the virulence properties of enhanced attachment/uptake and activation of the innate immune receptor, Toll-like receptor 2 (TLR2) [5].

8.2.1 Chlamydial Developmental Cycle

The biphasic developmental cycle of chlamydiae is unique among microorganisms and involves two highly specialized morphologic forms, both of which are required for infection and disease to occur: the infectious, extracellular form called an elementary body (EB) and the noninfectious but metabolically active intracellular form called a reticulate body (RB). The EB contains extensive disulfide cross-links both within and between outer membrane proteins giving it an almost spore like structure that is stable outside of the cell. The small infectious EB is inactive metabolically. Infection is initiated by attachment of EBs to the apical surfaces of epithelial cells of the conjunctiva, urogenital, or respiratory tracts, followed by receptor-mediated endocytosis. The EBs quickly modify their early endosomal membrane to exit the endosomal pathway, thereby avoiding fusion with lysosomes, and traffic on microtubules to the peri-Golgi/nuclear hof region. The EB-containing endosomes of *C. trachomatis* then fuse homotypically with one another to form their one nascent microcolony called an inclusion. The EBs then transform into RBs, the chromosome becomes relaxed and transcriptionally active, and metabolic growth and binary fission ensue to generate progeny. Chlamydiae-directed modification of their inclusion

membrane permits interception of trans-Golgi vesicles for transfer of sphingomyelin and glycerolphospholipids to the inclusion membrane, which can expand to accommodate some 200–1,000 progeny; this strategy of acquiring host cell markers for the inclusion also provides some degree of camouflage for the chlamydiae within. After 48–72 h, multiplication ceases and nucleoid condensation occurs as the RBs transform to new infectious EBs. The EBs are then released from the cell, allowing for infection of new host cells to occur.

The biphasic and relatively prolonged developmental cycle of chlamydiae are survival advantages. Antibiotic treatment or the host immune response must be able to kill both extracellular non-replicating infectious EBs as well as intracellular replicating RBs hidden within their protective vacuole if they are to rid the host of infection. Thus, antibiotic treatment requires multiple-dose regimens for 7–14 days. Single-dose azithromycin treats genital *C. trachomatis* infection effectively because it has a half-life in host cells of 5–7 days. The ability to cause prolonged, often subclinical infection is a major characteristic of chlamydiae.

8.2.2 Classification

Chlamydia trachomatis has been divided into subgroups based on antigenic variation in the major outer membrane proteins (serovars) and clinical expression. Microimmunofluorescence and monoclonal antibody testing have shown that there are more than 18 serovars of *C. trachomatis* with several distinctive clinical patterns of disease: trachoma is caused by serovars A, B, Ba, and C; oculogenital and neonatal disease by serovars B, Da, Ga, Ia, and D-K; and lymphogranuloma venereum (LGV) by serovars L1, L2, L2a, and L3. LGV infections are more invasive, as these serovars can replicate in macrophages, whereas replication of the other serovars of *C. trachomatis* is confined to mucosal epithelial cells.

8.3 Pathogenesis

8.3.1 Immunopathogenesis

In the realm of infectious diseases, it has often been observed that an overly aggressive inflammatory host response can be more problematic than the infection that initiated it. This is certainly true in the case of chlamydial infection, where the pathology that leads to the serious morbidities of chronic pelvic pain, ectopic pregnancy and infertility after female genital tract infection, is the result of the host inflammatory response. The *cellular paradigm* of chlamydia pathogenesis [6] states that the host response to chlamydiae is initiated and sustained by epithelial cells that are the primary targets of chlamydial infection. Infected host epithelial cells act as first responders, initiating and propagating immune responses through recognition of various chlamydial ligands via pathogen recognition receptors. They secrete chemokines that recruit inflammatory leukocytes to the site of infection, as well as cytokines that induce and augment the cellular inflammatory response and these mediators induce direct damage to the tissues. Unfortunately, this response is frequently ineffective at resolving infection, and ongoing stimulation of the host cells and bystander cells leads to continued release of tissue-damaging mediators. Since reinfection with chlamydiae is a frequent occurrence, repeated inflammatory responses may lead to repeated insult to the tissues, and promote further scarring. Since the host cell response to bacteria is the inciting inflammatory event, increased and prolonged bacterial burden correlates directly with disease development. Pathogen-specific and environmental factors that promote infection and bacterial survival lead to enhanced disease.

In vivo immunological studies in animal models and immune-epidemiological studies in humans indicate that resolution of infection can occur with minimal to no disease provided that the correct responses are induced in the right amount. Recognizing that the immune response to this organism leads to tissue damage, it is important to delineate the specific host

responses involved in disease promotion both for rational vaccine design and the discovery of biomarkers to monitor the effectiveness of candidate therapeutics and vaccines. Limited studies have been conducted in women investigating human genetic functional polymorphisms related to innate immune molecules. Investigation of polymorphisms in the gene for the innate immune receptor, TLR2 revealed single nucleotide polymorphisms associated with protection against tubal disease following *C. trachomatis* infection [7]. Interestingly, studies in the mouse model of female chlamydial genital tract infection have revealed activation of TLR2 is a key mechanism for induction of oviduct pathology [8]. Chlamydial induced activation of TLR2 leads to enhanced neutrophil influx, cytokine and protease production and prolongs neutrophil longevity [9]. Multiple reports indicate an important role for neutrophils and their products in oviduct tissue damage [10–13].

8.3.2 Immunoprotection

The adaptive immune response that occurs with chlamydial infection may lead to natural resolution of infection over time. However, the chronicity of infection in women indicates the suboptimal nature of the response. The natural course of *C. trachomatis* infection was described in a study of Columbian women followed for a 5-year period [14]. Eighty-two women found to be positive for *C. trachomatis* at the start of the study were studied at 6-month intervals. Most of the women (57.3 %) were >30 years of age (70.7 % were >25 years of age). Infection was classified as persistent if the same serotype was found at follow-up visits. Women who had taken antibiotics effective against *C. trachomatis* while infected were excluded. All study women reported 1–2 lifetime sex partners (82.9 % reported a single lifelong sex partner), thus the potential for repeated infection from an untreated male sex partner was high. Approximately 46 % of the infections were persistent at 1 year, 18 % at 2 years, and 6 % at 4 years of follow-up as determined by PCR of cervical scrape samples. Thus,

in nearly half of this female cohort, an adaptive immune response effective in eradicating their infection or in preventing repeat infection did not develop for up to 1 year.

The adaptive response is also suboptimal with respect to protection from reinfection. The high frequency of repeat infections found in clinic-based studies has led some authors to recommend screening female adolescents for *Chlamydia* as frequently as every 6 months [15]. However, there is a strong inverse relationship between age and susceptibility to chlamydial infection even when corrected for frequency of sexual contact, suggesting effective adaptive immunity eventually develops. Lymphoproliferative responses, but not serum antibody titers increase with age [16]. Data from humans point to MHC Class II–restricted CD4+ T cells of the Th1 phenotype as being critical to recovery from chlamydial infection as well as having a role in protection from disease [17, 18]. In a cohort of female commercial sex workers with HIV, susceptibility to chlamydial PID increased as numbers of CD4+ T cells decreased. Furthermore, in a prospective cohort study of commercial sex workers in Nairobi at high risk of exposure, production of IFN-γ by peripheral blood mononuclear cells stimulated with chlamydial antigen strongly correlated with protection against incident *C. trachomatis* infection [19].

Data from the mouse model of genital tract infection reveal that chlamydia-specific B cells and antibody effectively lower the bacterial burden upon challenge, thereby partially protecting the oviduct from infection and disease [20, 21]. Surface proteins including MOMP are likely the principal targets of neutralizing antibodies. Anti-chlamydial antibodies are not sufficient to protect humans from reinfection. In female sex workers levels of chlamydial EB-specific IgA and IgG detected in endocervical mucus and plasma were not significantly associated with a decreased risk of infection [19]. Although antibody may not play a primary role in protection from reinfection, it may help control the shedding of organisms and protect against upper tract disease. One study reported the prevalence of mucosal IgA antibodies was inversely related to the quantity

of *C. trachomatis* shed from the human endocervix [22], and another found the presence of serum IgA and IgG antibodies reduced the risk for ascending infection among women undergoing therapeutic abortion [23].

8.4   Epidemiology

Chlamydia trachomatis is the most common bacterial sexually transmitted infection, with an estimated 92 million cases occurring globally each year, including more than four million in sexually active adolescents and adults in the United States [24]. In reports from other parts of the world, the prevalence ranges from 28.5 % among female sex workers in Dakar [25], to 5.7 % among pregnant women in Thailand [26], and 0.8 % overall among women seen in private gynecology practices in Paris and 5.2 % for those under the age of 21 years [27].

In the US, substantial racial/ethnic disparities are present in the prevalence of both chlamydial and gonococcal infections. One large study of US female military recruits found a chlamydial prevalence of 9 % that was maintained over four consecutive years [28]. Young age, black race, home-of-record from the south, more than one sex partner, a new sex partner, lack of condom use, and a history of having a sexually transmitted disease were correlates of chlamydia infection.

Urine screening for chlamydial infection in Louisiana public schools revealed the overall prevalence of *C. trachomatis* was 6.5 %, with rates among girls more than twice that of boys (9.7 % vs. 4.0 %). The highest prevalence for boys occurred in 12th grade (8.9 %), whereas the highest prevalence for girls occurred in 10th grade (15.8 %) [29]. The high prevalence rates in this cohort are in contrast to a rate of 0.9 % for a cohort of 1,114 patients aged 15–24 years in two pediatric private practices in suburban North Carolina. In sexually active participants, prevalence was 2.1 %; in sexually active females, 2.7 %; and in sexually active males, 0.9 %. Most participants were female (63 %), white (87 %), and from highly educated families (64 % of their mothers graduated from college) [30].

Identification of persons with the highest risk of infection should enhance cost-effectiveness of screening and treatment programs. However, in a recent study among 3,202 sexually active adolescent females attending middle school health centers in Baltimore, MD chlamydial infection was found in 771 first visits (24.1 %) and 299 repeat visits (13.9 %); 29.1 % had at least one positive test result [15]. Unfortunately, independent predictors of chlamydial infection—reason for clinic visit, clinic type, prior sexually transmitted diseases, multiple or new partners, or inconsistent condom use-failed to identify a subset of adolescent females with the majority of infections.

8.5   Clinical Manifestations

Most studies report that 25 % of men and 70 % of women infected with *C. trachomatis* are asymptomatic or minimally symptomatic. The National Longitudinal Study of Adolescent Health Study collected data prospectively from 14,322 US adolescents and followed them into adulthood [1]. Of the participants that tested positive for chlamydial infection, 95 % did not report symptoms in the 24 h preceding specimen collection. Among men with chlamydial infection, the prevalence of urethral discharge and dysuria were only 3.3 % and 1.9 %, respectively. Among women with chlamydial infection, the prevalence of vaginal discharge and dysuria were 0.3 % and 4.2 %, respectively. Among the small number of young men reporting urethral discharge ($n = 17$), the prevalence of chlamydial infection was high (38.5 %), whereas the prevalence of chlamydial infection was only 0.9 % among women reporting vaginal discharge ($n = 98$). Of note, 6.0 % of the women reporting dysuria ($n = 232$) had chlamydial infection [1].

8.5.1   Infections in Males

When symptomatic, males frequently complain of dysuria or note a clear or mucopurulent urethral discharge at least 7–14 days following

contact with an infected partner [31]. The discharge may be so slight as to be demonstrable only after penile stripping and then only in the morning. Some patients may deny the presence of discharge but may note stained underwear in the morning resulting from scant discharge overnight. The primary complications of chlamydial urethritis in men are (1) epididymitis; (2) sexually reactive arthritis, including Reiter's syndrome; and (3) transmission to women. *Chlamydia trachomatis* and *N. gonorrhoeae* are the most frequent causes of epididymitis in men under age 35; urethritis also is usually present.

Although asymptomatic rectal carriage of *C. trachomatis* occurs in both infants [32] and adults, [33] *C. trachomatis* is a fairly common cause of proctitis and proctocolitis in men who have sex with men [34]. If the infection is due to a lymphogranuloma venereum strain, a severe proctocolitis can develop. Approximately 1 % of men with nongonococcal urethritis develop acute aseptic arthritis of presumed immune-mediated etiology [35]. One third of patients have the full complex of Reiter syndrome (arthritis, nonbacterial urethritis, and conjunctivitis); most such patients carry the histocompatibility antigen HLA-B27 [36].

8.5.2 Infections in Females

In women, chlamydial infections may cause PID, tubal infertility, chronic pelvic pain, and ectopic pregnancy. Chlamydial infection may also be linked to cervical cancer [37]. Chlamydial and gonococcal infections may increase susceptibility to and transmission of HIV in both men and women [38].

Symptoms in females include mild abdominal pain, intermittent bleeding, vaginal discharge, or dysuria-pyuria syndrome. The cervix can appear normal or exhibit edema, erythema, friability, or mucopurulent discharge. In prepubertal girls, vaginitis can occur secondary to infection of transitional cell epithelium by *C. trachomatis*. In contrast, the squamous epithelium of the adult vagina is not susceptible to chlamydiae, and vaginal discharge generally reflects endocervical infection.

Pelvic inflammatory disease is a sexually transmitted infection that ascends from the vagina and cervix to involve the uterus, ovaries, and peritoneal tissues as well as the fallopian tubes. Lower abdominal pain, usually bilateral, is the most common presenting symptom. Pain may be associated with an abnormal vaginal discharge, abnormal uterine bleeding, dysuria, dyspareunia, nausea, vomiting, fever, or other constitutional symptoms. It may also be present in a subclinical form that lacks the typical acute symptoms, but continues to lead to the associated long-term sequelae of infertility and ectopic pregnancy [39]. The most important causative organisms are *C. trachomatis* and *N. gonorrhoeae*; one or both of these agents cause well over half of cases. Other microorganisms implicated in PID include organisms found in the abnormal vaginal flora of women with bacterial vaginosis, such as bacteroides species, anaerobic cocci, *Mycoplasma hominis*, and *Ureaplasma urealyticum*. *Escherichia coli* and other enteric organisms have also been found.

A recent review of 24 studies examined PID diagnosis and sequelae after untreated chlamydial infection [40]. In one study, eighteen of 109 (16.5 %) asymptomatic adolescent women infected with *C. trachomatis* followed for 2 months or more became symptomatic, but only 2 (1.8 %) developed clinical PID [41]. On average in high-risk settings, 2–5 % of untreated females developed PID within the ~2-week period between testing positive for *C. trachomatis* and returning for treatment [42, 43]. However, the rate of progression to PID in the general, asymptomatic population followed up for longer periods appeared to be low [44]. The best data regarding the risk of PID over a long period of time is probably from the prevention of pelvic infection (POPI) trial conducted in the UK. Women in the control group had their vaginal swab stored. When analyzed 12 months later, 7 of 75 (9.5 %) women positive for chlamydial infection at baseline had developed PID [45].

The spectrum of PID associated with *C. trachomatis* infection ranges from acute, severe disease with perihepatitis and ascites (Fitz-Hugh-Curtis syndrome), to asymptomatic or "silent" disease. When women with chlamydial salpingi-

tis are compared to women with gonococcal or with nongonococcal-nonchlamydial salpingitis, they are more likely to experience a chronic, sub-acute course with a longer duration of abdominal pain before seeking medical care. Yet, they have as much or more tubal inflammation at laparoscopy [46]. In several studies, repeated chlamydial infection was associated with PID and other reproductive sequelae, although it was difficult to determine whether the risk per infection increased with each recurrent episode [17, 47]. No prospective studies have directly assessed the risk of infertility after untreated *C. trachomatis* infection. However, according to the largest studies, after symptomatic PID of any cause has occurred, up to 18 % of women may develop infertility [48].

8.5.3   Infections in Neonates

Neonatal infection generally is acquired during passage through an infected birth canal. Prospective studies of infants born to women with a chlamydial infection of the cervix have shown a 50–75 % risk that the infant will acquire *C. trachomatis* infection at one or more anatomic sites [49–51]. In exposed infants, risk of conjunctivitis is 20–50 % and risk of pneumonia is 5–20 %.

Ophthalmia neonatorum is the major clinical manifestation of neonatal chlamydial infection [52]. The usual incubation period is 5–14 days after birth, but symptoms can occur earlier after premature rupture of membranes or as late as 6 weeks after birth [53]. Typically, the most common presenting symptom described is a watery ocular discharge which becomes progressively more purulent (95 %), followed by swelling of the eyelids (73 %) and conjunctival erythema (65 %) [52]. The majority of chlamydial conjunctivitis resolves spontaneously during the first few months of life. However, if the condition is untreated, a chronic conjunctivitis can develop and persist for weeks or months. Although conjunctivitis may be quite severe, corneal ulceration, scars and pannus formation are rare and recovery is usual without visual impairment. Mild or subclinical infection can persist in some cases for years [54]. The neovascularization of

the cornea resulting from repeated infection in classic trachoma does not occur with neonatal disease.

Afebrile pneumonia caused by *C. trachomatis* in infancy occurs characteristically between 3 and 12 weeks of age, but may sometimes present later [55–57]. Characteristically, the infant has been symptomatic for three or more weeks before presentation. Most infants are only moderately ill and are afebrile. Symptoms of nasal obstruction and a pertussis-like, non-productive staccato cough gradually worsen over a week or more. Physical findings include tachypnea and rales but not wheezing. About 50 % of the affected infants have a history or evidence of conjunctivitis; a similar proportion has middle ear abnormalities [58]. Laboratory findings may include hyperinflation with symmetric interstitial infiltrates on chest radiography, peripheral eosinophilia (>400 cells/mm^3), and increased levels of serum immunoglobulins.

8.6   Laboratory Diagnosis

A positive laboratory test for *C. trachomatis* can be utilized for patient education and increases both compliance with drug therapy and the likelihood of referral of sexual partners. Although the development of tissue cell culture methods in the 1960s for detecting *C. trachomatis* was a major advance, the availability of nonculture tests has dramatically increased the availability and decreased the cost of laboratory detection. Definitive diagnosis of chlamydial infection, as would be required in a medicolegal setting (i.e., suspected sexual abuse or rape), requires isolation of *C. trachomatis* in cell culture or a positive nucleic acid amplification test (NAAT) confirmed by a second NAAT that targets a different sequence [59].

8.6.1   Diagnostic Specimens

Many screening tests for *C. trachomatis* require appropriately handled samples containing columnar epithelium from mucosal sites (e.g., endocervix, urethra, or conjunctiva) rather than

exudate; the adequacy of specimens should be verified by periodic cytologic evaluations. The discomfort caused by obtaining a urethral swab in males has precluded its widespread use in asymptomatic men. A dipstick test for leukocyte esterase performed on the first portion of a voided urine is a cost-effective and moderately sensitive screen (47–58 %) for detection of chlamydial infection in asymptomatic young males [60]. When feasible, urine NAAT provides a much more sensitive and equally noninvasive method of detecting *Chlamydia*.

8.6.2   Cell Culture

Use of chlamydial transport media containing antibiotics maximizes recovery and reduces the likelihood of culture overgrowth by other bacteria. Swabs used to obtain a specimen should have plastic or metal shafts, as soluble components from wooden shafts can have a toxic effect on cell cultures. Storage at −4 °C or maintenance at −70 °C is required if inoculation within 24 h is not possible. Cycloheximide-treated McCoy or HeLa cell lines are used most frequently to isolate *C. trachomatis.* Centrifugation techniques appear to enhance absorption of chlamydiae to cells. Intracytoplasmic inclusions can be detected at 48–72 h with species-specific immunofluorescent monoclonal antibodies for *C. trachomatis* and Giemsa or iodine stains. Generally, a higher isolation rate using cell culture is found in symptomatic patients than asymptomatic ones.

8.6.3   Nonculture Tests for *C. trachomatis*

NAATs amplify nucleic acid sequences that are specific for the organism being detected, and can detect as little as a single copy of target DNA or RNA. These tests have a higher sensitivity than all other tests, while retaining high specificity when cross contamination is being avoided [61]. NAATs have FDA approval for cervical swabs from women, urethral swabs from men, and urine from men and women [62, 63]. Similar to other nonculture tests, NAATs do not require viable organisms. NAATs detect *C. trachomatis* in urine or in self-administered vaginal swab specimens with sensitivity comparable to clinician obtained urogenital swab specimens, which makes non-invasive testing for chlamydial infections possible on individual as well as pooled specimens from a single patient [60, 64]. Multiple studies have determined that NAAT of self-obtained vaginal swabs are an acceptable, simple and sensitive diagnostic sample for the detection of *C. trachomatis,* as well as the sexually transmitted disease pathogens, *N. gonorrhoea* and *Trichomonas vaginalis* [65, 66]. Data suggest that NAATs are equivalent to or better than culture for the detection of *C. trachomatis* in the conjunctiva and nasopharynx of infants [67], and are currently being used in evaluation of newborns with conjunctivitis or pneumonia.

Enzyme immunoassays (EIAs) use enzyme-labeled chlamydial-specific antibodies to detect chlamydial LPS. The enzyme converts a colorless substrate into a colored product, which is detected by a spectrophotometer. EIAs are less sensitive than culture and NAATs; especially when using samples that contain few organisms (asymptomatic infections). EIAs lack specificity and will detect *C. pneumoniae* in respiratory specimens.

8.6.4   Serology

Antibodies to *Chlamydia spp.* are best detected with a microimmunofluorescent (MIF) assay, but these assays are not widely available. Serologic screening is of very little value in uncomplicated genital infections but may be useful for population studies. The MIF assay is species-specific and sensitive but is available only at a limited number of clinical laboratories.

8.7   Treatment

The most widely used treatments for uncomplicated oculogenital infections caused by *C. trachomatis* in nonpregnant adolescents and adults is doxycycline for 7 days or azithromycin in a

single dose [68]. In populations in which compliance with treatment is poor, azithromycin may be more cost-effective because it provides single-dose, directly observed therapy. Doxycycline costs less than azithromycin, and it has been used extensively for a longer period. Ofloxacin is similar in efficacy to doxycycline and azithromycin, but it is more expensive and offers no advantage with regard to dosage regimen. Erythromycin is less efficacious than both azithromycin and doxycycline, and gastrointestinal side effects discourage adherence to treatment.

Sex partners should be evaluated, tested, and treated if they had sexual contact with the patient during the 60 days preceding either diagnosis of *Chlamydia* or onset of symptoms in the patient. The most recent sex partner should be treated even if the time of the last sexual contact was >60 days before diagnosis of the index case. Patients do not need to be retested for *Chlamydia* after completing treatment with doxycycline or azithromycin unless symptoms persist or reinfection is suspected. A test of cure may be considered 3 weeks after completion of treatment with erythromycin. Testing at <3 weeks after completion of therapy to identify cases that did not respond to therapy may not be valid [69].

Azithromycin is currently recommended as first choice to treat *C. trachomatis* in pregnant women with amoxicillin as alternative [70]. Doxycycline and ofloxacin are contraindicated in pregnant women. Azithromycin is widely prescribed during pregnancy and lactation. Although it is excreted in breast milk, the dose delivered to the infant is quite low and not likely clinically significant [71]. Based on available data, the benefits of human milk feeding outweigh the risks of infant exposure to the small amounts of azithromycin transmitted through breastfeeding.

The optimal treatment for neonatal chlamydial conjunctivitis or pneumonia is uncertain. Erythromycin is recommended in many guidelines and has been the most widely-used antibiotic for neonatal chlamydial infection despite its association with infantile hypertrophic pyloric stenosis (particularly in the first 2 weeks of life). Azithromycin is an alternative but has not been well studied in this setting and it is also uncertain whether

there is a decreased risk of infantile hypertrophic pyloric stenosis with this macrolide.

8.8   Complications and Sequelae

Chlamydia trachomatis has been implicated as a pathogen in 8–54 % of women who have PID and has been associated with the long-term consequences of tubal infertility (17 %), ectopic pregnancy (10 %), or chronic pelvic pain (17 %) [72–74]. *C. trachomatis* infection has been associated with spontaneous abortion, though not consistently, and stillbirth [75–77]. Additionally, *C. trachomatis* infection has been associated with chorioamnionitis, premature rupture of membranes and preterm delivery. A 2.6 and 3-fold increased risk of preterm delivery were determined in women with positive serology detected at 17 weeks gestation or diagnosed with cervical infection at 24 weeks gestation, respectively [76, 78]. Evidence grows with the use of NAATs that preterm delivery is associated with *C. trachomatis* infection [79–81].

In males, epididymitis, prostatitis, and reactive arthritis are the most common sequelae. Furthermore, untreated or incorrectly treated chlamydial conjunctivitis may result in chronic conjunctivitis that can develop alone or as part of Reiter syndrome. Given the high prevalence of chlamydial infections, complications due to this pathogen account for serious morbidity and economic cost.

8.9   Prevention

Because chlamydial infections usually are not associated with overt symptoms, prevention of infection and screening of asymptomatic high-risk patients is the most effective means of preventing disease and sequelae. Behavioral interventions (i.e., delaying intercourse, decreasing the number of sex partners, and use of barrier contraception) should be pursued aggressively. High-risk patients who should be routinely tested for *Chlamydia* include women with mucopurulent cervicitis, sexually active women less than

20 years old, and older women with more than one sex partner during the last 3 months or inconsistent use of barrier contraception while in a nonmonogamous relationship [70]. Because of the frequency of repeated chlamydial infections within the first several months following treatment of an initial infection, [15] more frequent (e.g., every 6 months) screening of asymptomatic sexually active adolescents may be necessary. Clinicians and health-care agencies should consider advising all women with chlamydial infection to be rescreened 3–4 months after treatment. Providers are also strongly encouraged to rescreen all women treated for chlamydial infection whenever they next present for care within the following 12 months, regardless of whether the patient believes that her sex partners were treated. Screening and treatment programs have resulted in reduced rates of complications [82, 83].

In a recent study conducted in the UK, seven of 74 women randomized to deferred chlamydial testing and treatment developed clinical PID over a 12 month follow-up period (9.5 %) compared to one of 63 (1.6 %) treated at the time of enrollment [45]. Although this difference was not significant ($p = 0.07$) the annual incidence of PID (38 out of 2,377 women; 1.6 %) was less than the 3 % used in the sample size calculations, and thus the study was underpowered. In addition, participants were advised to be screened independently, and the one in five who acted on this advice had a high prevalence of chlamydial infection. Importantly, most cases of PID over 12 months occurred in women who were negative for chlamydia at baseline, indicating an importance for incident infection and the need to focus on testing those at higher risk, such as women with a new sexual partner.

In areas where screening and treatment programs have been established, rates of infection in screened populations have risen, causing speculation that early case identification and treatment interferes with development of immunity [84]. Others have insisted that the detection of increased rates of infection reflect a greater awareness of the infection, which has led to more testing being done, and still a larger number of positive tests [85]. Until a vaccine is developed to combat chlamydial disease, screening and treatment of infected persons remains the most logical mechanism of disease prevention.

To prevent maternal postnatal complications and chlamydial infections among infants, pregnant women should be screened for *Chlamydia* during the third trimester to permit completion of treatment before delivery. Ocular prophylaxis with topical erythromycin or tetracycline has reduced the incidence of gonococcal ophthalmia but does not appear to be effective against *C. trachomatis* [86]. Infants born to infected mothers are at high risk for infection; however, prophylatic antibiotic treatment is not indicated, and the efficacy of such treatment is unknown. Infants should be monitored and treated appropriately if symptoms develop.

8.10 Future Directions

During the extracellular EB stage, antibodies can act to inhibit infection. However, since the replicating RB form resides within the intracellular inclusion, bacterial killing at this stage requires a cell-mediated immune response with the primary effectors being IFN-γ secreting CD4 T cells. Thus, an ideal *C. trachomatis* vaccine should induce both local antibodies to prevent infection by EBs, and a strong Th1 response to limit infection once initiated. Efforts to develop a *C. trachomatis* vaccine have concentrated primarily on the use of recombinant *Chlamydia* antigens with immune adjuvants [87, 88]. The use of a purified native preparation of MOMP combined with Th1-inducing adjuvants induced significant resistance in mice, but sterilizing immunity was not achieved [89]. Stimulation of long-term mucosal immunity in the genital tract is a challenge; persons are susceptible to reinfection with *C. trachomatis* after a brief period of immunity because memory cells are not retained in the genital tract. It is unclear whether all genital infections could be prevented or whether only more invasive disease, such as salpingitis, might be preventable using vaccine technology. Markers for protection from upper genital tract infection and/or disease in the female will be nec-

essary if vaccine candidates are to be tested in humans.

Although current antibiotic treatment is highly successful when administered, most persons infected with *C. trachomatis* are asymptomatic and thus go undiagnosed and untreated. Although rates of infection have increased in certain areas where widespread screening and treatment programs have been in place [84], complications from infection have decreased [82, 83], indicating the utility of such programs. Public health officials should pursue such strategies in parallel with the ongoing research for effective vaccines.

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Treatment of Resistant Bacterial Infections in Children: Thinking Inside and Outside the Box

9

Gilat Livni and Shai Ashkenazi

Abstract

Antimicrobial resistance of bacteria causing pediatric infections has become more common and complicated in recent years. Although formerly confined to hospital settings, multi-drug resistant bacteria now also cause community-acquired infections. Treatment of infections caused by resistant pathogens is difficult, necessitating thinking both inside and outside the box. Determination of the precise minimal inhibitory concentration (MIC) is often crucial for selecting the most appropriate antibiotics, their doses, and use of prolonged infusions. For some multiply-resistant bacteria, off-label use of antibiotics, sometimes with no evidence from controlled studies ("salvage therapy") is unavoidable.

9.1 Introduction

9.1.1 Antibiotic Resistance

Though recognized for decades, bacterial resistance to antimicrobial agents has increased dramatically over recent years [1–4], as witnessed by several illustrative phenomena. First is the appearance of acquired multi-drug resistant bacteria, both Gram-positive bacteria that are resistant to multiple agents, including glycopeptides [5], and Gram-negative bacteria resistant to multiple agents, including carbapenems [1, 2, 6]. Resistance is mediated by complex and diverse mechanisms, which mainly include drug inactivation, target modification, target bypass, decreased outer membrane permeability, and increased efflux [6, 7].

Bacteria have devised varied and efficient mechanisms for the transfer of resistance-encoding genes among other bacteria, including those of different species, using genetic recombination and mobile elements, such as plasmids, integrons, and transposons [6, 8]. The result is the rapid dissipation of resistant bacteria among departments, institutions, countries, and continents [1, 2, 6, 9].

In the past, antibiotic resistance, and particularly multiply-resistant bacteria, was limited to

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Table 9.1 Stages of antibiotic therapy

1. Initial empiric antibiotics
Based on:
A. The suspected bacterial causes of the infection
B. The expected *in vitro* antibiotic susceptibility of these pathogens
C. Penetration to the site of infection
D. Results of clinical studies
E. Presence of contraindications or drug interactions
2. After 16–24 h: based on initial growth, but the final identification and antibiotic susceptibility are not yet available
3. After an additional 8–24 h: according to identification of the pathogen(s) and its antibiotic susceptibility

hospital settings, especially to intensive care units and to children with serious underlying health problems. The situation has changed, as demonstrated by increased rates in the last 10 years of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA), which now accounts for 70 % of all CA-*S. aureus* infections and 74 % of all *S. aureus* pneumonia in Texas Children's Hospital, USA [10]. Community-acquired Gram-negative bacteria that are carbapenem-resistant have also been reported [11]. This increasing and widespread bacterial resistance is occurring in an era when the options for new antimicrobial agents seem very limited [3, 12].

9.1.2 Approach to Antibiotic Therapy

Antibiotic therapy can be divided into three phases (Table 1). The first is the empiric antibiotic regimen, which is initiated when bacterial infection is suspected. Selection of specific antimicrobial agents is based primarily on the expected pathogens and their antibiotic susceptibilities, as well as on several other factors that are listed in Table 1. In positive bacterial cultures, initial growth of pathogens (i.e., of Gram-positive cocci or Gram-negative bacilli) is usually observed after 16–24 h, though definitive identification of the bacteria and their antibiotic susceptibility takes longer. Modifications in the antibiotic regimen may be required at this intermediary stage. Finally, after an additional 8–24 h, conclusive

identification of the pathogen(s) and their antibiotic susceptibility is generally possible. At this stage, "fine-tuning" of the antibiotic regimen is required to target the antibiotics according to the results from the microbiological laboratory.

"Direct susceptibility testing" is sometimes used, particularly for severe infections, to more rapidly attain antibiotic susceptibility. According to this approach, susceptibility testing is performed on the clinical specimen directly, without waiting for growth of a pathogen. As an example, due to limited penetration of most antimicrobial agents into the cerebrospinal fluid (CSF), the precise minimal inhibitory concentration (MIC) is crucial for optimizing treatment of bacterial meningitis. Therefore, in many institutions, if lumbar puncture reveals a purulent CSF, especially with a positive Gram stain, E-tests are placed directly on the CSF sediment, yielding the MIC within less than 24 h.

9.2 Treating Resistant Bacterial Infections in Children

The discussion of all resistant bacteria in children and their treatment options is obviously an impossible mission. We will focus therefore on major resistant bacteria causing pediatric infections and emphasize the most recent and clinically applicable data.

9.2.1 Penicillin-resistant Streptococcus pneumoniae

9.2.1.1 Pneumococcal Infections
S. pneumoniae is one of the most common pediatric pathogens, both in the community and in hospitalized children, causing mucosal (acute otitis media, mastoiditis, sinusitis, and pneumonia) and invasive (bacteremia, meningitis, septic arthritis, and osteomyelitis) infections [13]. More than 90 serotypes of *S. pneumoniae* have been described. Changes in the relative prevalence of the serotypes have occurred with and without relation to the conjugate vaccine use. For example, increased incidence of invasive pneumococcal disease caused by serotype 19A was

reported in the United States after introducing the pneumococcal conjugate vaccine in 2000, but an increase was also detected in other locations prior to vaccine use [13, 14].

9.2.1.2 Antibiotic Resistance

Resistance of *S. pneumoniae* to penicillin is caused by mutations in the penicillin-binding protein (PBP)-encoding genes [7, 14, 15]. These genes can spread by clonal dissemination of resistant strains, as well as by homologous recombination among different strains, either within pneumococci or with the closely related viridians streptococci. The modified PBPs have reduced affinity to penicillin and all other β-lactams. In addition, penicillin-resistant pneumococci often show resistance to other antimicrobial agents, including erythromycin, azithromycin, trimethoprim-sulfamethoxsazole (TMP-SMX), and tetracyclines [13, 14]. Tolerance of pneumococci to penicillin, mediated by changes in the peptidoglycan structure, has been reported, but its clinical significance is not completely clear. Based on recent pharmacokinetic/pharmacodynamic data, the Clinical and Laboratory Standards Institute (CLSI) updated in 2011 the susceptibility breakpoints for pneumococci [16].

9.2.1.3 Nonmeningeal Infections

Since pneumococci present reduced susceptibility but usually not full resistance, the principle of treating these infections is that high doses of β-lactams increase the fraction of time the antibiotic concentration in the area of the infection is above the MIC (fT>MIC), leading to microbiological and clinical efficacy [7, 13]. For nonmeningeal infections, the updated CLSI breakpoints of *S. pneumoniae* to parenteral penicillin are ≤2, 4, and ≥8 μg/ml, for susceptible, intermediate, and resistant strains, respectively [16]. Since MIC ≥8 μg/ml is very uncommon, most nonmeningeal infections (i.e., pneumonia, bacteremia) caused by *S. pneumoniae* can be treated successfully with high dosages (200,000–400,000 units/kg/day) of parenteral penicillin. Parenteral 3rd-generation cephalosporin (ceftriaxone or cefotaxime) may sometimes be indicated. Because of the high bioavailability, the 2011 breakpoints

for oral amoxicillin are the same as for parenteral penicillin [16]; for children who can take oral medication, high-dosage amoxicillin (80–90 mg/kg/d) is efficacious for most mucosal pneumococcal infections.

9.2.1.4 Meningeal Infections

Since the penetration of all β-lactams through the blood-brain barrier is very limited, the breakpoints for meningeal infections are much lower: ≤0.06, 0.12–1, and ≥2 μg/ml for parenteral penicillin, and ≤0.5, 1, and ≥2 μg/ml for 3rd-generation cephalosporins, for susceptible, intermediate, and resistant strains, respectively [16]. Determination of the MIC of the *S. pneumoniae* isolate causing meningitis is therefore crucial for optimal treatment: when the MIC to penicillin is < 0.1 μg/ml, high-dose parenteral penicillin is appropriate; when the MIC to penicillin is > 0.1 μg/ml but < 0.5 μg/ml to 3rd-generation cephalosporins, ceftriaxone or cefotaxime can be used; when the MIC to 3rd-generation cephalosporin is 1 μg/ml, vancomycin should be added to the ceftriaxone or cefotaxime; and when the MIC to 3rd-generation cephalosporins is ≥2 μg/ml, the addition of rifampin should be considered, especially if adjunctive steroid therapy is administered. For empiric treatment of bacterial meningitis in children, a 3rd-generation cephalosporin and vancomycin are recommended and the addition of rifampin should be considered if steroids are used.

9.2.2 Methicillin-resistant Staphylococcus aureus

Resistance of *S. aureus* to methicillin is mediated by mecA gene-encoding PBP2a with low affinity to methicillin, and in fact to all β-lactams (see below). Although methicillin is not currently in use, the term MRSA is still used to describe these strains [7, 10]. The expression of mecA genes can be constitutive or inducible; they are situated on mobile genetic elements called Staphylococcal Cassette Chromosome mec (SCCmec), which often contain genes coding for resistance to other antibiotics.

Table 9.2 Treatment options for multi-drug resistant Gram-positive infections

Antimicrobial agent	Comments
Vancomycin	The mainstay for resistant Gram-positive bacteria
Clindamycin	Mainly for CA-MRSA
Trimethoprim-sulfa-methoxazole	For SSTIs
Teicoplanin	Registered (not in the US) for SSTIs, bacteremia
Quinupristin-dalfopristin	A streptogramin, approved for SSTIs in > 16 year olds
Tigecycline	Glycycline antibiotic, inferior to β-lactams
Linezolid	An oxazolidinone antibiotic, excellent oral bioavailability
Daptomycin	A cyclic lipopeptide, not for pneumonia
Ceftaroline	5th-generation cephalosporin, approved for adults with SSTIs and community-acquired pneumonia

CA-MRSA community-acquired methicillin-resistant *Staphylococcus aureus*
SSTIs skin and soft tissue infections

9.2.2.1 Community-acquired MRSA

For many years MRSA were limited to hospital settings, and particularly to intensive care units. However, from recent reports, mainly from the United States [10], rates of CA-MRSA infections in children seem to be rising steadily, with 10–22 % carriage rates documented in healthy children. Such high rates of CA-MRSA have not been reported in all locations. Particularly in the U.S. pediatric CA-MRSA has been shown to be particularly severe, causing syndromes like bacteremia with septic shock and purpura fulminans, necrotizing pneumonia and empyema, severe orbital cellulitis, multiple-site osteomyelitis with deep vein thrombosis, pyomyositis, and necrotizing fasciitis. A study from the US Children's Hospital showed that, compared to CA-methicillin-sensitive *S. aureus* infections, CA-MRSA infections result in higher numbers of days with fever (3.9 vs 1.8), of hospitalization (12 vs 9), and with positive blood cultures (3.4 vs 1.5) [17].

In contrast to nosocomial MRSA, most isolates of CA-MRSA are susceptible to non-β-lactam antibiotics, like clindamycin, TMP-SMX, aminoglycosides, fluoroquinolones, and tetracycline [7, 10, 17], thus enabling broader treatment options. This is because most CA-MRSA is encoded by the relatively small type I SCCmec, which does not contain other resistant genes.

9.2.2.2 Treatment of MRSA

Guidelines for the treatment of MRSA were published in the United States [18] and in the United Kingdom [19]. We highlight first that for staphylococcal cutaneous abscess, incision and drainage comprises the primary management and is likely to be adequate alone. For example, in a double-blind, randomized, placebo-controlled study of 161 children with skin abscesses who underwent incision and drainage—no significant differences were noted between TMP-SMX and placebo after 10-day treatment or a 90-day follow-up call [20]. Systemic antibiotics are recommended for skin abscesses associated with the following conditions: severe local disease (multiple sites, rapidly progressed cellulitis, and septic phlebitis), systemic illness, comorbidities or immunosuppression, abscesses in difficult to drain areas (face, hands, and genitalia) and lack of response to incision and drainage alone [18].

The main antibiotic used for the treating of severe infections caused by MRSA is IV vancomycin. The recommended regimen for children with serious or invasive disease is 15 mg/kg/dose every 6 h, although data to support this dosing recommendation are limited [18]. In adults with serious infections (bacteremia, infective endocarditis, osteomyelitis, meningitis, pneumonia, and necrotizing fasciitis) due to MRSA, vancomycin trough concentrations of 15–20 μg/ml are recommended. The efficacy and safety of targeting similar concentrations in children are currently unclear [18].

Other treatment options exist (Table 9.2). Clindamycin is used mainly for CA-MRSA infections, because these strains are usually susceptible to this agent. It is recommended for children with osteomyelitis or septic arthritis who are stable, without ongoing bacteremia or intravascular infection. Initial treatment should be intravenous, with a dosage of 40 mg/kg/day divided into 3–4 doses; oral therapy can then follow. A review of the medical records of 39 children with CA-MRSA

treated with clindamycin (4 of them received additional antibiotics) showed recovery in all patients, except one child with undrained pyomyositis and septic thrombophlebitis [17]. Since clindamycin-resistant CA-MRSA has been reported in some locations, local resistance patterns should be monitored. Usually a 3 to 4-week antibiotic course is recommended for septic arthritis and a 4 to 6-week course for osteomyelitis; however recent data from controlled studies show that for uncomplicated infections shorter courses are acceptable [21]. TMP-SMX can be used for uncomplicated skin and soft tissue infections (SSTIs), as can tetracycline in children older than 7 years. Teicoplanin is not registered in the United States, but is recommended in the United Kingdom for the treatment of SSTIs and bacteremia [19].

Linezolid is the first agent of a new class of antibiotics, the oxazolidinones, to be approved for use in adults and children [22, 23]. It is mostly bacteriostatic, and has broad antimicrobial activity against Gram-positive bacteria, including resistant streptococci, MRSA, methicillin-resistant *Staphylococcus epidermidis* (MRSE), and vancomycin-resistant enterococci (VRE). Because of its excellent bioavailability, the oral and intravenous dosages are similar. In addition to the common, yet mild, adverse events of diarrhea, vomiting, and headache, hematologic (mainly thrombocytopenia) and neurologic complications have been reported, mainly in adults [23]. Summarizing seven randomized controlled trials (six with vancomycin and one with a cephalosporin) and four uncontrolled trials, Garazzino and Tovo concluded that linezolid is safe and efficacious in children with serious Gram-positive infections. They recommended that it should be "reserved for children who are intolerant to or fail conventional agents" [22]. The recommended regimen is 10 mg/kg/dose, IV or PO, three times a day in children under the age of 11 years and twice a day in older children. In our pediatric department, we also have good experience with linezolid, regarding tolerability, safety, and clinical efficacy.

Daptomycin is a novel cyclic lipopeptide, whose activity is based on disruption of membrane functions of Gram-positive bacteria [23]. The antibacterial bactericidal activity includes resistant bacteria, such as MRSA and VRE, and resistant streptococci, with demonstrated *in vitro* synergy with aminoglycosides, β-lactams, and rifampin. Daptomycin is approved for the treatment of SSTIs, bacteremia, and right-sided endocarditis. Since it is inactivated by alveolar surfactant, it should not be used to treat pneumonia. This antibiotic is shown to cause muscle pain and weakness, with elevated creatine kinase levels. Pediatric data are very limited. A retrospective review from Dallas Children's Hospital (U.S.) described the experience of 16 children (15 with invasive S. aureus infections (14 MRSA) and one with VRE) treated with daptomycin [24]. The addition of daptomycin to "conventional" antibiotics resulted in clinical improvement in all but two patients and in bacteriologic cure in six of seven children with persistent bacteremia; no adverse effects were attributed to the daptomycin therapy [24].

Ceftaroline is the first "fifth-generation" cephalosporin (others are in the pipeline) approved by the FDA, in October 2010, for adults with SSTIs and community-acquired pneumonia [25, 26]. Though its activity is similar to that of ceftriaxone, ceftaroline was designed to have a high affinity to PBP2a, and is accordingly active against MRSA, VRE, and resistant pneumococci. The most common adverse events are diarrhea, nausea, and rash. Clinical studies in children are currently in the planning stages.

Dalfopristin/quinupristin is a combination streptogramin that is active against MRSA, MRSE, resistant pneumococci, and vanA-mediated resistant *E. faecium*; it is not active against resistant *E. faecalis* [23]. The agent is approved for children over age 16 years and adults with complicated SSTIs.

9.2.2.3 MRSA with Reduced Vancomycin Susceptibility

Several levels of reduced susceptibility of *S. aureus* to vancomycin have been defined.

Vancomycin-resistant *S. aureus* (VRSA) is defined as MIC ≥ 16 μg/ml, and is usually mediated by vanA [16, 27]. Although very alarming, it has been reported in only a few (~ 7) patients. These strains typically appear after prolonged

vancomycin use, and are often resistant to other antimicrobial agents as well.

Vancomycin-intermediate *S. aureus* (VISA) is defined as MIC of 4–8 µg/ml, and is probably caused by cell wall thickening with reduced levels of peptidoglycan cross linking; it is uncommon and associated with previous vancomycin use [7, 16, 28].

Increased MIC ("vancomycin creep") reflects the trend of increased MIC of *S. aureus* to vancomycin, although still within the susceptibility range (MIC ≤2 µg/ml) [16]. In adults, an increase in MIC from 0.5–2 µg/ml has been shown to double clinical failure rates of staphylococcal infections [28], and an increase in MIC from 1 to 2 µg/ml of staphylococcal bacteremia to double the 30-day mortality rate [29]. Consequently, the recommended dosages of vancomycin for MRSA were increased [18], although elevated levels of vancomycin MIC were not observed in a study of pediatric MRSA isolates [30].

9.2.3 Extended-spectrum β-lactamases

Since first described in Germany in 1983, extended-spectrum β-lactamases (ESBLs) have spread worldwide, with increasing frequency among Gram-negative isolates [3, 4, 7]. These enzymes are viewed as new β-lactamases, as they hydrolyze the oxyimino- cephalosporins and monobactams [31]. They are inhibited by β-lactamase inhibitors, a property that assists their identification in the bacteriologic laboratory. Currently, >500 types of ESBLs have been reported: >160 in the TEM class, >140 in the OXA family, >100 in the SHV family, and >80 in the CTX-M class [7, 31]. The Amp C β-lactamase, which is found predominantly in *Enterobacter* sp and is often inducible and chromosomally-encoded, also reduces permeability by porin channel loss in the outer membrane.

ESBLs are most frequently present in *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Escherichia coli*, mediating their resistance to broad-spectrum cephalosporins [7, 31]; yet they have also been detected in other Gram-negative organisms, including typhoidal and non-typhoidal *Salmonella* sp, *Pseudomonas aeruginosa*, and *Proteus sp*. ESBL-producing organisms present mainly in pediatric, and especially neonatal, intensive care units.

Most ESBLs are encoded by large plasmids, and therefore can spread relatively easily among bacteria, also of different genera [3, 7, 32]. The plasmids can carry additional resistance genes, such as those mediating resistance to aminoglycosides and fluoroquinolones, leading to multi-drug resistant organisms with limited options for treatment [32].

Rates of Gram-negative bacteria that express ESBLs vary greatly by geographical region. Rates among *K. pneumoniae* tend to be highest. According to the European Antimicrobial Resistance Surveillance Network (EARS-Net), rates in 2008 were <5 % in Scandinavian countries, 25–50 % in Italy, Portugal, and Turkey, and >50 % in Greece. In the United Kingdom, the rate increased from 2 % in 2000 to 12 % in 2007 [32]. ESBL-producing organisms are usually associated with hospital-acquired infections, sometimes as outbreaks, and mainly in neonatal intensive care units. Previous exposure to antimicrobial agents, especially to ceftazidime, has been related to increased prevalence of ESBL-producing bacteria [7, 32]. These bacteria mostly affect children with comorbidities; most commonly causing bacteremia, septicemia, pneumonia, urinary tract infections (UTIs), wound infections, and ocular infections, with a greater risk of clinical treatment failure than for susceptible strains [32].

9.2.3.1 The Treatment of ESBL-producing Bacteria

Since ESBLs are inhibited by β-lactamase inhibitors, the combination of a β-lactam-β-lactamase inhibitor, such as piperacillin-tazobactam, is often used to treat infections caused by ESBL-producing bacteria; another possibility is an aminoglycoside, mainly amikacin [23]. A "thinking-outside-the box" approach uses prolonged β-lactam infusion to overcome the increased MIC of resistant Gram-negative organisms, ultimately optimizing the PK/PD of the available antibiotics

Table 9.3 Treatment options for multi-drug resistant Gram-negative bacteria

Antimicrobial agent	Comments
Ampicillin-sulbactam	Intrinsic activity against *A. baumani*
Fluoroquinolones	For ESBL-producing bacteria, *P. aeruginosa*
Colistin	For CRE, *A. baumani*, *P. aeruginosa*
Tigecycline	For CRE, *A. baumani*; inferior to β-lactams

ESBL extended-spectrum β-lactamase
CRE carbapenem-resistant Enterobacteriaceae

[33]. Since the efficacy of β-lactams is primarily determined by the fraction of time in which the drug concentration exceeds the MIC (fT > MIC), prolonging the infusion time results in more consistent serum levels and increases the fT > MIC. Prolonged infusion consists of either extended or continuous infusion; extended infusion is defined as intermittent infusion lasting ≥3 h, and continuous infusion as a 24-h fixed rate administration [33]. The clinical benefit of prolonged infusion has been demonstrated in adults, using piperacillin-tazobactam or ceftazidime, whereas studies in children are pending [33].

As the ESBL-encoding plasmids sometimes carry additional resistance genes, the resistance pattern of these organisms should be monitored in each medical unit, and the empiric antibiotic determined accordingly. In some cases fluoroquinolones are required, although they are not generally approved for individuals >17 years (Table 9.3). ESBLs are not capable of hydrolyzing carbapenems, which are usually the last option for the treatment of the ESBL-producing Gram-negative bacteria.

9.2.4 Carbapenem Resistance

9.2.4.1 Carbapenem Use in Children

Carbapenems are the mainstay in the treatment of pediatric infections caused by multi-drug resistant Gram-negative bacteria; due to increasing resistance, carbapenems are often the last resort for these infections [3, 23, 32]. All four carbap-enems—meropenem, imipenem, ertapenem, and doripenem—are administered parenterally, and their elimination is predominantly by glomerular filtration in the kidneys. They all have broad spectrum antimicrobial activity, though some differences exist among them [23]. Meropenem, which is the most active against Gram-negative bacteria, is most commonly used in neonates and children. Imipenem, which is more active against Gram-positive bacteria than is meropenem, is administered with cilastatin to avoid hydrolysis and to reduce nephrotoxicity. Seizures are an important adverse effect of imipenem, occurring in about 3 % of the treated patients who do not have central nervous system infections, and in rates reaching and exceeding 30 % in patients treated for bacterial meningitis [23]. Ertapenem demonstrates reduced activity against *Pseudomonas* isolates; in contrast, doripenem is most active against *P. aeruginosa*, but pediatric data are very limited. Carbapenems are not active against MRSA, *Stenotrophomonas maltophilia*, *Burkholderia cepacia*, *E. faecium*, *Mycoplasma* sp, and *Chlamydia* sp.

9.2.4.2 Resistance to Carbapenems

Resistance of Gram-negative bacteria to carbapenems is usually mediated by one of the following three mechanisms: impermeability due to porin channel loss in the outer membrane, alterations in PBPs, and carbapenemase-mediated hydrolysis [1, 3, 9]. Most common and alarming is the production of carbapemases [6, 9, 34]. The carbapenemases are divided into three classes (A, B, and D), and include *K. pneumoniae* carbapenemases (KPCs), several serine carbapenemases, and several metallo-β-lactamases [34]. KPC enzymes, classified as types 1–8, are no longer confined to *K. pneumoniae*, but have spread among other Gram-negative bacteria, including other *Klebsiella* sp, *E. coli*, *Salmonella enterica*, *Enterobacter* sp, *P. aeruginosa,* and others [1, 9, 34]. Outbreaks of KPC-producing bacteria have been reported in the United States, South America, Greece, Israel, India, and China [34].

Rapid dissemination of the New Delhi metallo-β-lactamase 1 (NDM-1) was recently reported [6, 11]. The bla$_{NDM-1}$-encoded gene was first detected in a Swedish patient who had been

hospitalized in New Delhi and was colonized with *K. pneumoniae* and *E. coli* containing this enzyme. The resistant gene was carried on plasmids of varying size, which readily transferred *in vitro* between bacterial strains. Indeed, this new type of metallo-β-lactamase spread rapidly to the United Kingdom and Pakistan, and subsequently to all continents [9]. The bla$_{NDM-1}$-encoded mobile gene disseminated into diverse bacteria, including *Enterobacter* sp, *Proteus* sp, *Citrobacter* sp, and *A. baumani*. Infections caused by these resistant organisms comprised septicemia, pneumonia, urinary tract infections, SSTIs, and diarrhea, with many fatal cases [6, 9]. The observation that some of these infections caused by the resistant bacteria were community-acquired is very alarming [9].

9.2.4.3 Treatment

Treatment options for multi-drug resistant Gram-negative bacteria are listed in Table 9.3. Ampicillin-sulbactam has intrinsic activity against *A. baumani*, and is used for infections caused by multiply-resistant *A. baumani* [7]. The fluoroquinolones, which inhibit DNA replication by inhibiting the topoisomerase, have broad antimicrobial activity, mainly against Gram-negative organisms [23]. They are active against ESBL-producing bacteria and *P. aeruginosa* [23, 35]. Pediatric effectiveness has been shown for meningitis, infections in children with cystic fibrosis, complicated urinary tract infections, bacterial diarrhea, and chronic suppurative otitis media. The major drawback, however, is that the fluoroquinolones induced changes in the immature joint cartilage of all laboratory animals studied. In children, reversible arthralgia or arthritis has been documented, but no clear permanent arthropathy. The fluoroquinolones are therefore not approved for use in children, except for in unusual settings; ciprofloxacin, for example, is approved for use in children in many countries for limited indications. Fluoroquinolone use in children is only recommended when an alternative antimicrobial therapy is not feasible [23, 35].

Colistin, Polymixin E, is a large cationic polypeptide that binds to the bacterial anionic lipopolysaccharide and increases membrane permeability, ultimately leading to cell death. This polypeptide is active against most aerobic Gram-negative bacteria, including those resistant to other antibiotics, such as multi-drug resistant *A. baumani* and *P. aeruginosa* [36]. Colistin has concentration-dependent bactericidal activity, with a considerable post-antibiotic effect. Colistimethate, a prodrug that is hydrolyzed to the bioactive colistin, is the commercial intravenous formulation, which can be administered also by inhalation. While generally not absorbed from the gastrointestinal tract, it has been administered orally for bowel decontamination, as well as topically for bacterial skin infections. It does not cross the blood-brain barrier; case reports of intrathecal or intraventricular use for resistant Gram-negative bacteria have been described. Excretion is mainly by glomerular filtration. Because of the major concern of nephrotoxicity during colistin therapy, its IV use was abandoned in the 1980s. Recent data from adults, however, indicate that colistin-mediated nephrotoxicity is less prevalent than previously thought, occurring in 8–19 % of treated patients, mainly as tubular damage [34]. Neurotoxicity presented in about 7 % of the patients, and allergic reactions in about 2 %.

Colistin is typically used as "salvage therapy" for bacteria that are resistant to carbapenems and other antibiotics (Table 9.3).

Tamma and Lee reviewed colistin use in children [36]. Of 271 children without cystic fibrosis who were treated with colistin, 86.5 % were cured; nephrotoxicity was noted in 2.8 %, and neurotoxicity was not observed. A retrospective study evaluated colistin use for the treatment of septicemia in a tertiary neonatal intensive care unit during 2009 [37]. Eighteen critically ill neonates who received colistin and at least one additional antibiotic were identified. A favorable clinical response was observed in 76 %, and microbiological clearance in 81 %, with no major adverse events. The authors concluded that IV colistin appears to be safe and efficacious in neonates, including preterm babies [37].

Tigecycline is the first glycylcycline antibiotic, a new class of antimicrobial agents, that have been approved in many countries for the treatment of complicated SSTIs, complicated intra-abdominal

infections, and community-acquired pneumonia in adults [38]. It is active *in vitro* against a wide-range of resistant bacteria, including MRSA, carbapenem-resistant Enterobacteriaceae, and *A. baumani*. The dosage in children above 8 years old is 1 mg/kg every 12 h. A recent systematic review and meta-analysis identified 15 randomized controlled trials that examined the efficacy of tigecycline. Compared with other antibiotic regimens, tigecycline therapy was associated with higher clinical failure, development of septic shock, microbiological failure, and overall mortality [38]. The authors concluded that "clinicians should avoid tigecycline monotherapy in the treatment of severe infections and reserve it as a last-resort drug" [38]. Accordingly, the US Food and Drug administration (FDA) issued a warning regarding the use of this drug.

9.3 Conclusions

Increasing rates of multi-drug resistant bacteria have recently been witnessed, with some organisms resistant to all currently available antimicrobial agents [2, 3, 6, 11]. In a world in which travel is very common, resistant bacteria spread rapidly [6]. In an era with very limited options for new antimicrobial agents [12], our ability to effectively treat bacterial infections in adults and in children is threatened [11]. Immediate action is needed, especially to decrease the spread of resistant bacteria. A recent study demonstrated the success of a nationally implemented broad infection control program in containing a nationwide outbreak of carbapenem-resistant *K. pneumoniae*; monthly numbers of new cases of resistant strains decreased by 79 %, as detected by active surveillance [39]. This evidence of the feasibility of controlling antibiotic resistance gives hope for the efficacy of antibiotics in the future.

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An Encephalitis Primer

10

Dominic Kelly

Abstract

For most clinicians managing a patient with encephalitis will be an infrequent event. The range of differential diagnoses in a child presenting with a clinical syndrome that could be encephalitis is broad. In addition, there are many pathogens that can cause encephalitis. In view of these facts, a systematic approach to clinical assessment, investigation and treatment, based on epidemiology and an understanding the limits of investigations, is crucial to effective management of this condition. This review focuses on encephalitis aetiology and investigation; HSV encephalitis; mycoplasma encephalitis; and flavivirus encephalitis as a globally important cause of disease.

10.1 Introduction

Encephalitis presents several challenges to the clinician. Firstly. the presenting signs and symptoms of central nervous system (CNS) disease (e.g. altered consciousness/behaviour, seizures, focal neurology) and infection (e.g. fever, rash) are common to a wide variety of non-infectious and infectious conditions which are hard to distinguish on clinical grounds alone. Secondly, the pathogens able to cause encephalitis are numerous. Finally, at presentation children are usually becoming progressively more unwell and

may have significant neurological and respiratory compromise for which timely intervention and support will prevent secondary damage.

In view of these facts a systematic approach to clinical assessment, investigation and treatment, based on epidemiology and an understanding the limits of investigations, is crucial to the effective management of this condition. This short review will focus on encephalitis aetiology and investigation, HSV encephalitis (due to its prevalence and the existence of treatment), mycoplasma encephalitis (frequently implicated in encephalitis but for which there are significant uncertainties around pathogenesis and treatment), flavivirus encephalitis (as a globally important cause of disease) and 'Louping ill' (out of local interest).

Several comprehensive reviews of childhood encephalitis have been published recently [1, 2]

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and recommendations on investigation and management produced in the US and UK [3, 4].

10.2 Regional Epidemiology Together with a History of Travel, Immunisation Status, Immune Compromise and Animal Exposure Guides Initial Investigation

Although viral causes are most common, encephalitis or encephalitis-like syndromes can also be caused by bacteria, mycobacteria, fungi and parasites.

UK and North American studies demonstrate the importance of the following pathogens in childhood encephalitis (treatable causes are underlined) [5–7]:

1. Herpes viruses (HSV, VZV, EBV)
2. Respiratory viruses (e.g. influenza, para-influenza, adenovirus)
3. Enteroviruses
4. *Mycoplasma pneumoniae*

A history of travel, lack of immunisation, immune compromise and animal exposure extends this list to include:

5. Lack of immunisation—Measles, mumps
6. Travel—Arboviruses (e.g. Japanese encephalitis, West Nile virus, tick borne encephalitis; St Louis encephalitis)
7. Immunocompromise—HIV, Listeria, Toxoplasma, CMV, HHV6, JC, Cryptococcus
8. Animal exposure—*Borrelia burgdorferi*, Rabies, *Bartonella henselae* (Cat-scratch disease)

HSV herpes simplex virus, *VZV* Varicella Zoster virus, *EBV* Epstein Barr virus, *HIV* human immunodeficiency virus, *CMV* cytomegalovirus, *HHV6* human herpes virus 6

Other pathogens may cause clinical syndromes very similar to encephalitis and these, although usually considered separately, are an important consideration in the differential diagnosis of treatable causes. Such pathogens include; bacteria causing meningitis (or a meningo-encephalitis) such as *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae*; *Plasmodium falciparum* causing malaria; *Mycobacterium tuberculosis* causing TB meningitis.

The relative importance of these pathogens varies by region of the world and season [8]. In central, northern and eastern Europe, tick borne encephalitis is the a prevalent pathogen associated with encephalitis. In areas of South-East Asia, such as Vietnam, Japanese encephalitis predominates. Such is the importance of tick borne encephalitis and Japanese encephalitis in these regions that in some countries, vaccines against these pathogens are part of the routine immunisation schedule.

Whilst not exhaustive, the above list, combined with a full history, allows investigation for the pathogen causing encephalitis to be focused on the most likely organisms. However it is important to note that even with extensive investigation no definite pathogen is identified in a significant proportion of cases [5, 9, 10].

Of the many organisms causing encephalitis only a limited number have specific treatment (these are highlighted in the list above). Despite the prevalence of arboviruses in many regions of the world there is currently little to support specific treatment for any of these organisms. There is a single case report of an individual surviving rabies associate with a specific intensive care unit based treatment protocol but this is the exception and has not been replicated [11].

10.3 Initial Investigation and Empirical Treatment should be Focussed on Non-infectious Differential Diagnoses Together with Common or Treatable Infectious Causes

The extent of investigation will depend on resources available and region. Confirmation of encephalitis is based on the combination of findings from cerebrospinal fluid (CSF) microscopy, CNS imaging, identification of a specific pathogen (e.g. HSV from CSF PCR) and in some cases EEG. The importance of investigation for non-infectious causes lies in the lack of specificity of the initial presenting clinical syndrome and the wide variety of other treatable causes (Table 10.1). Various guidelines have been published and the list of first-line investigations

Table 10.1 Non-infectious differential diagnoses for children presenting with possible encephalitis. (Adapted from Long 2010 [2]) (With kind permission from Springer Science + Business Media B.V.)

Non-infectious, para-infectious and autoimmune	Reye syndrome
	Acute disseminated encephalomylitis
	Acute necrotizing encephalopathy
Neoplasia	Primary or metastatic
	Paraneoplastic diseases
Cerebrovascular	Ischemic stroke
	Subdural/epidural hematoma
	Vasculitis
Systemic	Metabolic conditions
	Connective tissue disorders
	Drug intoxication
Other	Epilepsy
	Head injury
	Confusion migraine

used in one UK centre, for the child presenting with encephalopathy which may be encephalitis, is given here (Table 10.2).

Empirical anti-microbial treatment should include cover for likely bacterial pathogens causing CNS infection. In the UK, penicillin would cover *N. meningitidis* and most *S. pneumoniae*, however the possibility of *H. influenzae*, *Staphylococcus aureus*, penicillin resistant *S. pneumoniae* and Gram negative pathogens means that a third generation cephalosporin (eg ceftriaxone) is indicated. For the severely unwell child it is reasonable to cover *Listeria* with amoxicillin until the clinical picture is clearer as, although rare, it is described in immunocompetent children. High dose intravenous acyclovir treatment is mandatory for any child with suspected encephalitis. Other treatments depend on the specific history and risk factors for the individual case.

10.4   HSV Encephalitis is Treatable but Still Problematic

HSV is the commonest non-seasonal cause of encephalitis (HSVE) and is treatable. Despite this HSV often provides the clinician with diagnostic

and therapeutic dilemmas. The outcome of HSVE in the pre-acyclovir era was devastating, with a mortality of 70 % and neuro-disability almost the rule amongst survivors. In 1986 a comparison of 10 days of acyclovir demonstrated a significantly improved outcome in comparison to vidarabine [12]. Of 69 individuals recruited to the trial the mortality in the 32 randomised to acyclovir treatment was 28 % versus 54 % in the vidarabine group. Of the survivors, 38 % in the acyclovir group versus 14 % in the vidarabine group were 'functioning normally'. In these trials the duration of treatment was 10 days and there were no relapses. However one of the authors of this study noted that 'many patients were still febrile at the conclusion of treatment suggesting that a longer duration of therapy …may be advisable' [13]. Relapses of patients treated with acyclovir have been reported and in addition neonatal trials indicated the improved outcomes of 21 days of treatment versus 10 days (although the latter group also had a lower dose of acyclovir) [14]. These factors have led to the current recommendations for treatment duration of 14–21 days. The major modifiable factor associated with poor outcome in HSVE is now delay in starting acyclovir and clinicians should have a low threshold for starting acyclovir in any child with a compatible syndrome.

Detection of HSV DNA on PCR testing of CSF is the current 'gold standard' of diagnosis. However a negative CSF PCR is found in a number of children in the early stages of disease (see Table 10.3). Diagnostic work-up should therefore include an LP at least 72 h after the onset of symptoms even if this means a repeat LP for children who presented at < 72 h. An important issue is that a number of children with subsequently proven HSV will have a normal CSF white cell count [15]. Whilst scanning is usually abnormal in HSVE, this may not be the case early in the course of the illness particularly with CT [16]. A wide variety of imaging findings have been described from the classic fronto-temporal lesions through to more diffuse lesions. The decision to discontinue acyclovir treatment in a child with an encephalitis-like illness needs to be considered in the light of these diagnostic uncertainties and should be based on clinical recovery

Table 10.2 The investigation of children with unexplained encephalopathy. **a** Initial investigation and **b** targeted further investigation in relation to risk-factors from detailed history. (Adapted from clinical guidelines at the Children's Hospital, Oxford)

Investigation	
Radiology	: CT (+/- contrast) or MRI if available
	Sedation contraindicated - contact anaesthetist for GA if necessary
Blood	: FBC/film, UEs/glucose/Ca/Mg, LFTs/coags
	Blood gas, **ammonia (urgent),** lactate, amino acids (biochemistry 'to store')
	Blood culture
	Serology (>2ml) for EBV, CMV, Mycoplasma, Lyme, HIV (specific consent required)
CSF	: Opening pressure, gram stain, culture, gluc/protein, lactate, PCR (HSV/VZV/Entero/Adeno)
Urine	: Culture, organic/amino acids/toxicology (biochemistry 'to store')
Throat swab	: PCR (Entero/Adeno)
NPA	: PCR (Influenza/Parainfluenza/Adeno/RSV/Entero) only if compatible symptoms

a

Box 1 Additional ID investigations

Many uncommon infectious causes of encephalopathy have specific treatments(box 3) and are important to consider in terms of history of potential risk factors (e.g. travel, animal exposure, immune compromise, immunisations (MMR)) +/- investigation including;

Immunocompromise

CMV	- blood/CSF pcr
HHV6	- CSF pcr
Measles (unvaccinated)	- serology (serum/saliva)
Cryptococcus neoformans	- Cryptococcal antigen (blood/CSF), India ink stain (CSF)
Mycobateria tuberculosis	- CSF for AFBs/TB culture
Toxoplasma gondii	- serology

Travel – country-dependent

Malaria	- thick and thin film
Rickettsial diseases (Typhus, spotted fevers)	- serology
Rabies if animal bite/ bat exposure	- discuss investigation
Arboviruses (JE, WNV, TBE)	- serology / CSF pcr
Measles (unvaccinated)	- serology (serum/saliva)
Mycobaterium tuberculosis (contact history)	- CSF for AFBs/TB culture

Animal exposure

Bartonella henselae (cat-scratch)	- serology
Rabies if animal bite/ bat exposure	- discuss investigation

b

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together with (i) normal MRI, (ii) negative CSF PCR at >72 h post-onset of symptoms and negative EEG [1]. In practice if all of these investigations are normal and there is complete recovery then discontinuing acyclovir around 10 days may be considered in that the risk of HSVE is relatively low and a course of treatment has been given that was shown to be efficacious in the original trials. If there are significant clinical, CSF, MRI or EEG abnormalities consistent with HSVE, and no alternative diagnosis, then a full 21-day course of treatment is warranted despite a negative CSF PCR [1, 17]. An algorithm to facilitate the decision to discontinue acyclovir treatment has been described previously [17] and this issue is discussed in detail in a recent review of childhood encephalitis [1].

With sensitive CSF PCR testing, cases of possible HSVE may be identified in clinical settings where the illness appears very mild compared to

Table 10.3 Case series which document the occurrence of initial negative HSV PCR in individuals with subsequent confirmed herpes simplex encephalitis. [15, 39–41]

Study	Total number with HSE	Initial negative HSV PCR	
Weil et al. [41] Clin Infect Dis	11	3	Negative day 1–3, positive day 4–7
Stugahl et al. [40] Scan J Infect Dis	10	2	Positive 4–7 days later
Guffond et al. [39] Clin Infect Dis	22	1	Negative day 3, positive day 8
De Tiege et al. [15] Clin Infect Dis	38 (children)	8	Negative day 0–3

typical HSVE or there are possible alternative diagnoses [18]. Whilst it has been speculated that some of these may be reactivation, of what is a persistent neurotropic virus, identification of HSV in CSF remains a clear indication for a full course of acyclovir treatment.

The availability of valaciclovir, a highly bioavailable alternative to intravenous acyclovir, has prompted some discussion about its role in treating invasive HSV disease. This is particularly relevant where a prolonged course of intravenous treatment requires secure venous access which in some cases can become more challenging as treatment progresses. There is a case report of oral valaciclovir use in one child with confirmed HSVE during the last week [19] and an uncontrolled series of cases from Vietnam treated with oral valaciclovir [20]. In the latter six individuals with confirmed HSVE were treated with oral valaciclovir and two died (days 2 and 3) whilst the other four completed treatment. These four had negative CSF PCRs by day 10 and CSF aciclovir concentrations were considered to be therapeutic, although levels fell with increasing duration of treatment. At present this agent cannot be recommended at any stage of treatment of HSVE.

Even with acyclovir there are still significant sequelae to HSVE and adjunctive treatments have been considered. Steroids have been associ-ated with clinical recovery in some case reports [21] and improved outcomes in some case series [22]. A randomised trial is currently in progress in Europe [23]. At the present time corticosteroids remain of unproven benefit in HSVE.

10.5 *Mycoplasma pneumoniae* is Frequently Identified in the Setting of Encephalitis

M. pneumoniae is a common respiratory tract infection in childhood and is associated with a wide variety of neurological complications including encephalitis. In some studies of encephalitis *M. pneumoniae* has accounted for 5–13 % of cases [24, 25]. It's relative rarity in other studies may relate to limitations of current diagnostic methods [26] and also the occurrence of disease in cycles (3–4 years in the UK) longer than most epidemiological studies [27]. The detection of organisms via culture or PCR in CSF suggests that direct invasion of the central nervous system plays an important role in pathogenesis in at least some individuals [24, 26]. However immunological mechanisms are likely to be important in many children including cases of ADEM [28]. In most centres testing is limited to serology whose limited specificity and sensitivity reduces the ability to determine the role of this agent in encephalitis. In one of the most detailed case series, PCR identified *M. pneumoniae* DNA in CSF, or in throat swabs in conjunction with positive serological evidence of infection, in almost 7 % of cases of childhood encephalitis (11 out of 159 children) [24]. Around two-thirds of these children had significant neurological sequelae at follow-up. In a further 25 % of cases of encephalitis there was evidence for a possible role of *M. pneumoniae* although many of these children also had other pathogens detected as well. Not all children had a respiratory prodrome and the lack of such a prodrome was associated with the detection of *M. pneumoniae* by PCR in the CSF (rather than confirmation by serology with a negative CSF PCR). Given the evidence regarding neurological sequelae it is reasonable to consider empirical treatment for

M. pneumoniae in settings and seasons where infection is common. A CNS penetrating agent should be used and options include azithromycin [29], fluoroquinolones [30] and doxycycline although there is no definitive evidence for efficacy [3].

10.6 Arboviruses Including Japanese Encephalitis are Rarely Seen in the UK but are Globally Important Causes of Viral Encephalitis

Arbovirus (Arthropod Borne virus) is a colloquial term referring to viruses that are sustained in cycles of transmission between host vertebrate species and vector arthropods such as mosquitoes or ticks. A number of these viruses can cause encephalitis in humans, who are usually not the primary host but an evolutionary 'dead-end' for the virus. The major families of 'arboviruses' are Bunyaviridae (La Crosse virus), Togaviridae (e.g. Eastern and Western Equine encephalitis and Chikungunya) and Flaviviridae (e.g. Japanese encephalitis, West Nile virus, St Louis encephalitis, tick borne encephalitis). Globally it is viruses from the genus flavivirus, within the family Flaviviridae, which are responsible for the greatest burden of disease. The combination of vector ecology, host ecology and virus biology result in very specific geographic and seasonal patterns for these infections and the most significant flaviviruses vary by region. The mosquito-transmitted flaviviruses that typically cause encephalitis are Japanese encephalitis, West Nile virus and St Louis encephalitis. Although only a minority of infections result in significant disease, encephalitis is one of the characteristic disease syndromes of these viruses [31].

Japanese encephalitis is estimated to cause up to 67,900 cases of encephalitis annually [32] despite the fact that there is an effective vaccine which has been incorporated into routine immunisation programmes in many areas of the world. This virus is endemic in many areas of Asia and causes sporadic disease in the western Pacific and north Australia. Cases are predominantly in children, with significant neurological morbidity

and a case fatality rate of 20–30 %. Between 30–50 % of survivors suffer neuropsychological sequelae. Disease in returning travellers is rare. Whether this is due to exposure or immunisation of travellers is unclear but the short incubation period (5–15 days) reduces the probability of Japanese encephalitis being a significant consideration except in the first 1–2 weeks after return. The prevalence of this disease in certain regions has driven attempts to find an effective treatment including trials of steroids and interferon-2 alpha but to date none have been shown to have efficacy [33, 34]. Effective immunisation programmes of individuals exposed to infection is the mainstay of prevention for this disease. For many years Japanese encephalitis was known as Japanese B encephalitis although the use of this term has now mostly lapsed. The origin of this term was as a distinction from type A epidemic encephalitis (encephalitis lethargica).

Since the introduction of its mosquito vector into the US in 1999 West Nile virus has become an important diagnostic consideration in the North America [35]. The arrival of this pathogen to the USA demonstrates the dynamic epidemiology of this virus family related to the interdependence of host, vector and virus. Despite suggestive experiments in animal models with immunoglobulin treatment and some human data there remains no proven treatment for infection with this virus [36].

Some other flaviviruses rely on tick vectors. In many areas of central and eastern Europe and across Russia into China one of several strains of tick borne encephalitis make up one of the most common aetiologies of encephalitis [37]. The incidence of disease has been increasing over recent years perhaps in response to climatic variables. Several countries in this region include vaccines against this virus in routine immunisation schedules.

10.7 'Louping ill': the UK Flavivirus

Many regions of the world have syndromes of viral encephalitis related to arboviruses sustained in life-cycles adapted to very specific

local ecologies and the UK is no exception. The 'Louping ill' virus is the only flavivirus endemic in the UK [38]. It is closely related to tick borne encephalitis virus. The life-cycle involves tick vector (*Ixodes ricinus*) which is found mainly in areas of rough upland grazing or moorland. 'Louping ill' is an infection of the nervous system and occurs mostly in sheep and grouse. Human cases are rare and usually related to occupational exposure (abattoir workers, shepherds, vets). A range of syndromes have been described one of which is an encephalitis. There is no specific treatment and the name relates to the leaping behaviour of the sheep which develop symptomatic disease manifest by 'spring(ing) into the air.'

10.8 Conclusion

For the clinician, managing a child with an encephalitis-like syndrome a detailed history (including travel, immunisation status, animal exposure and evidence of immune-compromise) will facilitate prompt and effective investigation and treatment of important causative pathogens. Despite this, in a significant number of cases no aetiology will be identified. Key issues in management are prompt empiric treatment with antibiotics and acyclovir, consideration of other likely treatable causes (infectious and non-infectious) and good supportive care.

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The Evidence Behind Prophylaxis and Treatment of Wound Infection After Surgery

11

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Abstract

Surgical site infections (SSIs) represent a serious post surgical complication. They are the leading cause of healthcare-related infections in developing countries and the second most common healthcare-related infection in developed countries. Here we discuss the epidemiology of and risk factors for SSIs together with the current evidence supporting the use of antibiotic prophylaxis for the prevention of wound infection after surgery.

11.1 Introduction

In the early nineteenth century, Ignez Semmelweis demonstrated that washing hands in the obstetrical clinic resulted in a dramatic reduction in the rate of puerperal sepsis, and then he published a book "Etiology, Concept and Prophylaxis of Childbed Fever" of his findings [1]. Afterwards, Louis Pasteur and Joseph Lister revolutionized the concept of wound infection prevention using antiseptic procedures in surgery. Antibiotic prophylaxis was established in the 1960s, when it was demonstrated that antibiotics cause maximum suppression of infection if given before bacteria gain access to tissue [2]. Since then, many advances have developed to prevent and control wound infection after surgery.

Because of the increasing number of operative procedures, and despite strict infection control policies, SSI remains a leading cause of morbidity and mortality in modern health care settings. It is the second most common type of health care–associated infection (HAI) after urinary tract infection in developed countries, while it is the leading cause of HAI in developing countries [3, 4]. SSI occurs in 2–5 % of patients undergoing inpatient surgery in the United States [3, 5]; and its reported prevalence varies between 1.13 infections per 100 procedures in developed countries to 5.6 per 100 surgical procedures in developing countries, of which 48 % are complex SSI [4–6]. Previously published reports have shown that patients who develop SSIs, in comparison to those with non-infected surgeries, are up to 60 % more likely to spend time in the ICU, five

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times more likely to be readmitted to the hospital, and have a twofold increase risk of death [7]. In addition, 77 % of deaths in patients with SSI are attributed directly to SSI [8].

Infection of surgical wounds is defined as infection following a surgical procedure that occurs within 30 postoperative days, if no implant was placed, and up to 1 year if an implant was placed [8]. SSI is classified according to the degree of microbial contamination into the following [8, 9]:

a. Clean: Closed, uninfected wound with no evidence of acute inflammation. This includes an uninterrupted viscus (respiratory, gastrointestinal, biliary, or urinary tracts) during a clean procedure.
b. Clean contaminated: Elective entry of a viscus (respiratory, gastrointestinal, biliary, or urinary tracts) under controlled conditions with minimal spillage, and no evidence of infection under aseptic conditions.
c. Contaminated: Includes gross spillage from gastrointestinal tract, open accidental wounds, and major breaks in aseptic conditions. Usually associated with acute, nonpurulent inflammation.
d. Dirty: Includes preoperative perforation of viscera with retained tissue or foreign material, or fecal contamination, or presence of old penetrating traumatic wound. Usually associated with the presence of purulent inflammation.

The rate of SSI increases significantly from 7.6 % episodes per 100 surgical procedures in clean wounds to 39.2 episodes per 100 surgical procedures in dirty wounds [4]. In addition, the rate of SSI varies widely by the type of surgical procedure. It is highest with intra-abdominal operations followed by cardiovascular surgeries [10].

11.2 Aetiology of SSI

Infection of surgical wounds is mainly caused by the patient's endogenous flora, and it is believed that infection is acquired at the time of surgery by direct inoculation. The most common single pathogen contributing to SSI is *Staphylococcus aureus*, causing 20–37 % of all SSI [5, 11, 12]. Of these, 49–54 % are *methicillin-resistant S. aureus* (MRSA) strains; making MRSA the single most common pathogen isolated from SSI in community hospitals, with an increasing trend over recent years [4, 5, 11]. *Gram negative bacilli (Escherichia coli, Pseudomonas aeruginosa, Enterobacter species, Klebsiella species, Acinetobacter species and Proteus mirabilis)* are also considered major players, causing just under half of all SSIs [4, 13]. Other organisms causing SSI include *Coagulase negative staphylococci, Enterococci, Streptococci, Candida species,* and *anaerobic organisms* [5, 11, 13]. Infection with endogenous gut flora when surgery involves opening a viscus, and the infection is usually polymicrobial. Exogenous contamination of the wounds can rarely be acquired from the operating room personnel or the operation room environment [14, 15].

11.3 Risk Factors for SSI

Patient-related risk factors (such as diabetes, obesity, smoking, and known colonisation with resistant organisms) are well known predisposing risk factors for SSI. These, together with the surgical characteristics (introduction of foreign material, amount of tissue damage, duration of surgery, shaving) and pathogen characteristics (degree of contamination, microbial burden and virulence) form a complex relationship posing patients to higher likelihood of SSI [12, 13, 16]. Thus many important factors should be considered perioperatively to minimize the risk of surgical site infection. These include prophylactic antimicrobial administration, use of aseptic surgical techniques and proper surgical site cleaning, proper hair removal using clippers if required and the avoidance of shaving, cessation of smoking 30 days before surgery, ensuring proper oxygenation during surgery, blood sugar control and avoiding hyperglycemia in patients undergoing cardiac surgeries, and avoidance of

hypothermia in patients undergoing colorectal surgeries [8, 12, 13, 17–24]. While all are important, the current evidence behind prophylactic antimicrobial administration in surgical patients will be discussed in more details in the following section.

11.4   Perioperative Antimicrobial Prophylaxis

The use of perioperative antimicrobial prophylaxis is a proven intervention to reduce the risk of SSI in elective surgical procedures [25–28]. This aims to reduce the burden of possible pathogens at or in close proximity to the surgical incision at a critical time. The main principles that should be followed to maximise the benefit of antimicrobial prophylaxis include [8, 13] (i) using antimicrobial prophylaxis in elective surgeries with a high risk for infection or if SSI would have a high risk of deleterious outcomes, (ii) using a prophylactic agent that is safe, inexpensive, and bactericidal with activity against the most probable infective pathogens in the surgical procedure, (iii) timing the infusion of the antimicrobial agent so that a bactericidal concentration of the drug is present in serum and tissue at the time of incision and, (iv) maintaining a therapeutic serum and tissue level of the antimicrobial agent throughout the operation and until the incision is closed.

11.4.1   Indications for Perioperative Antimicrobial Prophylaxis

The National Institute of Health Service and Clinical Excellence (NICE) in the United Kingdom recommends antimicrobial prophylaxis to be given before clean surgeries that involve placement of a prosthesis or implant, clean-contaminated surgeries, and contaminated surgeries [29]. Antimicrobial prophylaxis for surgery should be used if evidence from clinical trials is available, in which the surgery requires entry into a viscus or implantation of a prosthetic

device, and in operations where SSI would pose major consequences [8, 17]. However, a recent meta-analysis demonstrated that prophylactic antimicrobial therapy is significantly associated with reduced risk of post-operative wound infection in 23 different types of surgeries, but the relative risk of wound infection did not vary between the different levels of surgery cleanliness; suggesting that prophylactic antimicrobial use is effective in reducing the risk of wound infection for all types of surgery including the ones with no available evidence from clinical trials [25]. Yet, the use of antimicrobial prophylaxis is associated with cost, potential adverse events, and possible development of antibiotic resistance; so this has to be well considered when using prophylactic antibiotics in clean surgeries.

Cardiothoracic surgeries are generally considered as clean surgeries, but they usually involve placement of implants. In addition, deep infection after cardiac surgeries is usually associated with catastrophic outcomes. Antimicrobial therapy in the settings of dirty wounds is considered part of the treatment because infection is already established [29]. In surgeries involving total hip and knee replacement, 13 patients need to be treated with antimicrobial prophylaxis in order to prevent one case of SSI [27].

For surgical intra-abdominal infections (IAIs), the current Canadian practice guidelines recommend antimicrobial prophylaxis in clean-contaminated abdominal surgeries for colorectal resection; small intestinal surgeries; oesophageal surgeries (obstruction, dilation, or sclerotherapy for varieces); high risk gastro-duodenal surgery (cancer, active bleeding, low gastric acidity, obstruction, obesity); high risk biliary-tract surgeries (acute cholecystitis, cholelithiasis or obstructive jaundice, open billiary-tract surgeries, old age, diabetes or obesity); and perforated, gangrenous or necrotising appendicitis [17]. However, a meta-analysis on antimicrobial prophylaxis for prevention of postoperative infection after appendectomy recommended prophylaxis in all patients undergoing operation for appendicitis with no apparent difference in the nature of the removed appendix [30].

Table 11.1 Suggested prophylactic antimicrobial regimens according to the type of surgery and infective pathogens

Type of surgical procedure	Infective pathogen	Prophylactic agent	Adult dose[a]	Timing of the infusion[b]	Duration (h)	Comments
Cardiac surgery [12, 31, 32, 37, 38, 44, 47]	*S. aureus* and CONS[c]	Cefazolin or Cefuroxime	1–2 g IV, 1.5 g IV	Within 60 min before incision	24–48	Vancomycin as an acceptable alternative if a prosthetic material is used
						β-lactam or penicillin allergy: vancomycin+aminoglycoside
						High risk for MRSA infection[d]: vancomycin (1 g IV)+cephalosporin
						If an aminoglycoside is given, do not repeat the dose
Non-cardiac thoracic surgery [8, 12, 31, 32]	*S. aureus*, CONS, *Streptococcus pneumonia*, and GNB	Cefazolin or cefuroxime	1–2 g IV, 1.5 g IV	Within 60 min before incision	≤24	High risk for MRSA infection[d]: vancomycin (1 g IV)+aminoglycoside
Abdominal surgery: [8, 12, 17, 28, 31, 32, 42, 54]						
Esophageal, gastrodudenal or biliary tract surgery	GNB, Gram positive cocci	Cefazolin	1–2 g IV	30–60 min before incision	≤24	β-lactam or penicillin allergy: clindamycin with either an aminoglycoside or a fluoroquinolone, High risk for MRSA infection[d]: vancomycin (1 g IV)+aminoglycoside
Colorectal surgery	GNB, *Enterococci*, and anaerobes	(IV): Cefazolin+, metronidazole or cefoxitin or cefotetan or ampicillin/ sulbactam	1–2 g IV, 500 mg IV, 2 g IV, 1–2 g IV, 3 g IV	30–60 min before incision	≤24	
Appendectomy	GNB, and anaerobes	(PO): Neomycin+erythromycin base	1–2 g IV, 500 mg IV, 2 g IV, 1–2 g IV, 3 g IV	30–60 min before incision	≤24	
		Cefazolin+, metronidazole or cefoxitin or cefotetan or ampicillin/ sulbactam				
Orthopaedic surgery [8, 12, 31, 32, 45, 48, 49, 55–57]	*S. aureus*, CONS, *Streptococci*, and GNB	Cefazolin or cefuroxime or teicoplanin	1–2 g IV, 1.5 g IV, 10 mg/ kg IV	No tourniquet: 30 min before surgery, Tourniquet:10 min before surgery	≤24	Vancomycin as an acceptable alternative if a prosthetic material is used, β-lactam or penicillin allergy: vancomycin or clinamycin [58] IV)
Urologic surgery [8, 12, 31, 32]						
Open surgeries or laparoscopy	GNB and *Enterococci*	Cefazolin	1–2 g IV	Within 60 min of incision	≤24	High risk for MRSA infection[d]: vancomycin (1 g IV)+aminoglycoside
Cystoscopy[e] or upper urinary tract instrumentation	GNB and *Enterococci*	Ciprofloxacin or TMP/SXT	500 mg PO or 400 mg IV, 1 DS tablet			

Table 11.1 (continued)

Type of surgical procedure	Infective pathogen	Prophylactic agent	Adult dose[a]	Timing of the infusion[b]	Duration (h)	Comments
Neurosurgeries [12, 31, 32, 59]	*S. aureus*, CONS	Cefazolin or cefuroxime	1–2 g IV, 1.5 g IV	Within 60 min of incision	≤24	High risk for MRSA infection[d]: vancomycin (1 g IV)

GNB Gram-negative bacilli, *CONS* coagulase-negative staphylococci, *MRSA* Methicillin-resistant *S. aureus*, *TMP/SXT* Trimethoprim/Sulfamethoxazole

[a]Repeat dose intraoperatively if prolonged surgery more than 4 h (every 3–4 h for cefuroxime and cefazolin, every 8 h for vancomycin and metrronidazole, and every 6 h for clindamycin)

[b]Two hours are allowed for the administration of vancomycin and fluoroquinolones. If vancomycin is used in cardiac surgeries, the dose should be administered within 60–16 min prior to incision

[c]Gram-negative bacilli are rarely reported in SSI after cardiac surgery, contamination occurs during the saphenous vein harvesting [60]

[d]High risk for MRSA infection: known MRSA colonization or coming from facilities with high prevalence of MRSA infection

[e]This includes any cystoscopy with manipulation, or high risk cystoscopy with positive urine cultures, transrectal prostatic biopsy, or preoperative urine catheter

11.4.2 Choice of Prophylactic Antimicrobial Regimens

The choice of prophylactic antibiotic regimen depends on the nature of surgery and the infective pathogens likely to cause the infection. In general, the chosen prophylactic agent should be safe, cost-effective, have good tissue penetration and bactericidal against expected pathogens [8, 17].

Table 11.1 summarises the recommended prophylactic antimicrobial regimens according to the type of surgery and the likely infective pathogens. In intra-abdominal surgical procedures, antimicrobial prophylaxis should include coverage for *S. aureus, Gram-negative bacilli* and *anaerobes* from the distal gastrointestinal tract [17, 31]. First generation cephalosporin (cefazolin) is effective in most procedures [31]. Metronidazole should be added in distal surgical procedures to cover anaerobic organisms [17, 28, 31]. Alternative regimens include cefoxitin, cefotetan or ampicillin/sulbactam alone [31, 32]. A recent meta-analysis on antimicrobial prophylaxis in colorectal surgeries demonstrated that the addition of aerobic coverage to anaerobic coverage and vice versa both resulted in statistically significant improvements in SSI rates, which supports the current recommended regimens [28]. Interestingly, the study also demonstrated that SSI was significantly lower when giving combined oral and intravenous antibiotic prophylaxis compared to intravenous alone, or oral alone [28]. The use of mechanical bowel preparation is currently not recommended before colorectal surgeries, as there is lack of evidence to support its use in preventing postoperative infectious complication, and it can be associated with rare but serious complications [33].

A meta-analysis of 28 placebo-controlled trials of cardiothoracic prophylaxis demonstrated that second-generation cephalosporins (cefamandole and cefuroxime) resulted in an approximate one and one-half-fold lower rate of SSI compared to cefazolin [26]. However, subsequent trials failed to discriminate between the different cephalosporins [34–36]. Because of this and the fact that Cefazolin has better activity against Staphylococci, as well as its availability and lower cost, it is the recommended prophylactic antimicrobial agent in cardiac surgeries [37].

In the era of community-associated MRSA (CA-MRSA), patients at high risk for MRSA infection (known MRSA colonisation or coming from facilities with high prevalence of MRSA infection) should receive perioperative vancomycin prophylaxis for prevention of MRSA infection [17, 37, 38].

Decolonisation with nasal mupirocin 5 days before surgery was examined in many presurgi-

cal patients. A meta-analysis showed that mupirocin prophylaxis in nasal *S. aureus* carriers was associated with significantly lower rate of nosocomial infections due to *S. aureus* among surgical patients, but secondary analysis restricted to SSIs only showed non significant results. The rate of infections caused by microorganisms other than *S. aureus* was also significantly higher in the treatment group. The authors suggested that in people who are nasal carriers of *S. aureus*, the use of mupirocin ointment results in a statistically significant reduction in *S. aureus* infections [39]. Another meta-analysis supported mupirocin use in non general surgery cases (e.g., cardiothoracic surgery, orthopedic surgery, and neurosurgery), but no benefit was found in general surgical cases [40]. Until rapid screening tests for *S. aureus* colonisation are widely available, mupirocin is currently recommended as a routine prophylactic measure for all patients undergoing cardiac surgical procedures [37]. Mupirocin use for decolonisation is still a controversial topic, as increasing incidence of mupirocin resistance is a potential issue. Moreover, it is not clear how many surgical patients need to be treated with prophylactic mupirocin in order to prevent one case of SSI, and it is not clear if prophylactic mupirocin should be administered to all pre-surgical patients or only to those colonised with *S. aureus*.

11.4.3 Timing of Perioperative Prophylactic Antimicrobial Infusion

The timing and dosing of the antibiotic infusion should be adjusted to attain peak serum and tissue concentrations at the critical moment of incision [17]. In 1992, Classen et al. demonstrated in a prospective study of 2,847 subjects that the lowest rates of SSIs occurred in the group of patients who received antimicrobial prophylaxis within 2 h of incision [41]. Afterwards, Weber and colleagues examined in a prospective cohort study the rate of SSI by the timing of surgical prophylaxis after cefuroxime (and metronidazole in colorectal cases) infusion in 3,836 surgical proce-

dures. They found that the most effective time for prophylactic antimicrobial infusion is between 30 and 60 min before surgery, while there was a significantly higher odds of SSI when pre-operative antimicrobial prophylaxis was administered less than 30 min (adjusted OR = 1.95; 95 %CI, 1.4–2.8; $p < 0.001$), or between 60 and 120 min (adjusted OR = 1.74; 95 %CI, 1.0–2.9; $p = 0.035$) before surgery [42]. The association between the prophylaxis timing and the occurrence of SSI was also assessed prospectively in a multicenter study involving 4,472 randomly selected cardiac, hip/knee arthroplasty, and hysterectomy cases. Results showed that the best protection was seen when the antibiotic was given within 30 min of incision [43].

The effect of timing of the prophylactic vancomycin infusion on the incidence of SSI was evaluated in 2,048 patients undergoing cardiac bypass graft or valve replacement surgery. Patients who received vancomycin 16–60 min before the beginning of surgery had a lower rates of postoperative infection than those who received vancomycin 0 and 15 min minutes preoperatively. Reduction in the rate of SSI was also noticed among those who received vancomycin 16–60 min before surgery compared to the ones who received it 61–120 min, 121–180 min, and more than 180 min before surgery, but this reduction was not statistically significant [44].

Administration of perioperative antibiotics 30–60 min prior to incision is the current recommended timing in the Canadian practice guidelines for surgical intra-abdominal infections [17]. The Society of Thoracic Surgeons Practice Guidelines recommend prophylactic antibiotics to be administered in cardiac surgery patients within 60 min of skin incision [37]. This timing is also recommended by the Surgical Infection Prevention Project that is developed by the Centers for Medicare & Medicaid Services in collaboration with the Centers for Disease Control and Prevention [32].

In aseptic orthopaedic surgeries, prophylactic antibiotics should administered within 30 min before incision and at least 10 min before tourniquet inflation [45]. Development of SSI does

not differ significantly if the prophylactic antibiotic is given before inflation of the tourniquet or shortly after inflation of the tourniquet [46].

11.4.4 Duration and Frequency of Perioperative Antimicrobial Prophylaxis

Therapeutic serum and tissue levels should be maintained throughout surgery and ideally until closure of the incision; thus in cases of prolonged surgical procedures (more than 3–4 h), prophylactic antibiotics may need to be readministered intraoperatively [17, 31]. Additional intraoperative doses are to be given at intervals 1–2 times the half-life of the drug, with the exception of aminoglycosides, when the dose should not be repeated [24, 31, 37]. In the absence of an established infection, or bowel perforation, or a penetrating bowel trauma operated within 12 h, antimicrobial prophylaxis should be limited to 24 h or less [17].

A recent meta-analysis of 12 studies involving 7,893 adult patients undergoing open heart surgery compared short-term (<24 h) with longer-term antibiotic prophylaxis (≥24 h). Longer-term antibiotic prophylaxis reduced the risk of sternal SSI by 38 % (risk ratio 1.38, 95 % confidence interval (CI) 1.13–1.69) and deep sternal SSI by 68 % (risk ratio 1.68, 95 % CI 1.12–2.53), with no significant differences in mortality, infections overall and adverse events. This suggests that perioperative antibiotic prophylaxis of ≥24 h may be more efficacious in preventing sternal SSIs in patients undergoing cardiac surgery compared to shorter regimens [47].

It was demonstrated in a meta-analysis of antimicrobial prophylaxis in colorectal surgery that there is no advantage to longer antibiotic dosing [28]. Sub-group analysis of three studies that specifically compared a single preoperative dose of antibiotic to either a second intraoperative dose, or early postoperative dose, or both, also showed no advantage with extended dosing. This suggests that a single dose of antimicrobial prophylaxis is equivalent to multiple perioperative doses in the prevention of SSI, which questions the recommendation of giving a second dose in longer operations [28].

The use of a single dose of antimicrobial prophylaxis was shown to be effective in many other trials. A prospective randomised study evaluated the efficacy of single versus multiple doses of teicoplanin as antimicrobial prophylaxis for arthroplasties in 616 patients. Single dose teicoplanin was found to be more effective as prophylaxis for total hip or knee arthroplasty compared with multiple doses [48]. Another study also demonstrated no difference in the rate of postoperative SSI after clean orthopedic surgery when comparing single dose versus multiple doses of prophylactic antibiotics [49]. Furthermore, data from 23 studies that included 8,447 subjects undergoing surgery for closed fracture fixation showed that single dose antibiotic prophylaxis was as effective as multiple doses in reducing the rate of deep SSI [50].

11.5 Outcome of Antimicrobial Prophylaxis

The Surgical Infection Prevention and the Surgical Care Improvement Projects aim to decrease the morbidity and mortality associated with postoperative surgical site infections. The project's antimicrobial prophylaxis performance measures suggested that (i) prophylactic antimicrobial should be given within 1 h of surgical incision (or within 60–120 min for fluoroquinolones and vancomycin); (ii) prophylactic antimicrobial choice should be consistent with published guidelines; (iii) prophylactic antimicrobials should be discontinued within 24 h of surgery [32]. Hospitals that improve compliance with the different components of appropriate antimicrobial prophylaxis reported decrease in the rates of SSI [51, 52]. The Surgical Infection Prevention Project performed a large study that included 35,543 surgical cases from 56 hospitals on the impact of improved infection control and antimicrobial prophylaxis process measures. Implementation of these measures resulted in a 27 % reduction in the average rate of SSI in the first 3 months after surgery [51]. In contrast, non adherence to the Surgical

Site Infection Prevention Guidelines in elective general surgical, neurological, and orthopedic procedures with more than two errors in antibiotic prophylaxis measures was significantly associated with increased rate of SSI (odds ratio 4.030; 95 % CI, 1.02–15.96) [53]. Furthermore, implementing a comprehensive infection control program for prevention of SSI after cardiac surgery demonstrated that prophylactic antimicrobial administration was a protective factor against deep sternal SSI [52].

11.6 Summary

SSI is a leading cause for healthcare associated infections worldwide. As discussed in this review, SSI can be prevented by implementing the recommended infection control measures. Perioperative antimicrobial prophylaxis is one of the most well studied measures with proven benefits in preventing SSI. The current recommendations are to provide prophylactic antibiotics according to the recommended guidelines. The chosen antibiotic should have activity against the pathogens likely to be encountered in the procedure. The prophylactic antibiotic should be administered within one hour of surgical incision (within 30–60 min in general surgeries), and be discontinued within 24 h of the surgery. An exception is in cardiac surgeries, for which most guidelines recommend discontinuation 24–48 h after the surgery.

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Infectious Risks Associated with Biologics

David Isaacs

Abstract

Biologics are generally either custom-designed monoclonal antibodies against specific target cells (e.g. B-cells) or target cytokines (e.g. tumour necrosis factor, TNF) or they are receptor constructs (fusion proteins) based on naturally-occurring cytokine or cell receptors. Biologics are mostly used in adult rheumatology but are increasingly used in paediatrics. There are significant concerns about safety and also about cost. The main safety concerns are about increased risk of infection and malignancy.

The use of TNF antagonists is associated with increased risk of serious infections with intracellular organisms, particularly mycobacteria, but also intracellular bacteria, fungi and Pneumocystis. B-cell antagonists like rituximab can cause progressive multifocal leukoencephalopathy. IL-6 antagonists are associated with increased rates of common bacterial infections and the complement pathway antagonist eculizumab with meningococcal infection.

The risk of some infections associated with biologics can be reduced, by screening patients starting TNF antagonists for latent tuberculosis and giving them cotrimoxazole prophylaxis against Pneumocystis, and by immunising against VZV, hepatitis B, meningococci and pneumococci. However, the risk of the biologics causing serious infection in children is unknown and needs study. Children should not be started on the biologics without careful consideration of the risks and without fully informed consent.

12.1 What is a Biologic?

The US FDA has a Center for Biologics Evaluation and Research which regulates a diverse array of complex products they term biologic agents [1]. The term biologics (or biologicals [2]) can be used to include a wide range of medicinal

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products including vaccines, blood and blood components, somatic cells, gene therapy, tissues, and recombinant therapeutic proteins created by biological processes (as distinguished from chemistry). Biologics can be composed of sugars, proteins, or nucleic acids or complex combinations of these substances, or may be living entities such as cells and tissues.

However, in most cases, the term biologics is used more restrictively for a class of genetically engineered medications produced by means of biological processes involving recombinant DNA technology. These medications are usually one of three types:

1. Recombinant human proteins. Examples are erythropoetin, growth hormone and biosynthetic human insulin and its analogues (all hormones).
2. Monoclonal antibodies. Custom-designed antibodies (using hybridoma technology or other methods) designed to counteract or block specific targets of the inflammatory response, either target cells (e.g. B-cells) or target cytokines (e.g. tumour necrosis factor or TNF).
3. Receptor constructs (fusion proteins), usually based on a naturally-occurring receptor for a cytokine (e.g. etanercept targets the TNF-alpha receptor) or for a cell receptor (e.g. belatacept blocks T-cell activation by targeting the CD 28/CTAL-4 receptor).

All of the currently used biologics have to be administered parenterally, either sub-cutaneously or by intravenous infusion, anything from daily (anakinra) to every few weeks, with obvious implications for the paediatric population. Most data come from use in adults, but paediatric use is increasing [2].

The biologics, when used in this sense, have arguably had most impact in adult rheumatology and notably in the treatment of rheumatoid arthritis, giving rise to the term biologic disease-modifying agents for rheumatic diseases or bDMARDs. However, they are being used increasingly in a range of fields, to treat inflammatory conditions (e.g. TNF-alpha inhibitors like infliximab for inflammatory bowel disease), malignancy (e.g. trastuzumab or Herceptin for breast cancer), and to treat transplant rejection (e.g. monoclonals directed at B-cells like rituximab). Almost every discipline now uses biologics to treat one or more disease with an immunologic contribution to pathogenesis.

However, the advent of biologic therapeutics has also raised significant concerns about safety and cost. The cost of biologic therapies is dramatically higher than the cost of conventional pharmacological medications. Furthermore, biologics are mainly used to treat chronic conditions such as rheumatoid arthritis or inflammatory bowel disease, or for the treatment of refractory cancer for the remainder of life. Safety concerns include increased risk of malignancy, particularly lymphoma, and worrying reports of demyelination, hepatotoxicity and severe allergic reactions to the biologics. This paper, however, will concentrate on the infectious risks of the biologics.

For all the biologics, the exact degree of increased susceptibility to infection is difficult to quantify. Randomised controlled trials and meta-analyses tend to consider "all infections", "serious infections" and "death" as their outcomes [3]. Most patients on biologics are also taking or were taking corticosteroids, methotrexate and other medications with profound effects on the immune system. Other important variables include underlying disease state, age, and prior and future exposure to pathogenic organisms such as mycobacteria, *Pneumocystis* and fungi.

12.2   Biologics and Infection

Biologics target the immune system. We know that patients with congenital or acquired immune deficiency are at increased risk of infections. Persons with severe congenital or acquired T-cell immune deficiency are also at increased risk of malignancy, particularly lymphoma. It is far from surprising, therefore, that biologics have been shown to be associated with increased risk of infections and of lymphoma.

What should intrigue infectious disease physicians is that different biologics are associated with different patterns of susceptibility to different infectious agents. Relating the nature of

Table 12.1 Biologics acting as inhibitors of tumour necrosis factor

Generic name	Trade name	Indications	Technology
Adalimumab	Humira	Rheumatoid arthritis, ankylosing spondylitis, psoriasis, Crohn's	Humanised monoclonal antibody
Etanercept	Etebrel	Rheumatoid arthritis, ankylosing spondylitis, psoriasis	Recombinant human TNF-receptor fusion protein
Infliximab	Remicade	Rheumatoid arthritis, ankylosing spondylitis, psoriasis, Crohn's	Monoclonal antibody
Golimumab	Simponi	Rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis	Monoclonal antibody

the infections caused by a particular biologic to the target of the biologic should help increase our knowledge of the normal human defences to infection.

A corollary of the previous statement is that there are some congenital immune deficiency diseases which affect a relatively specific part of the immune system and that knowledge of the nature infections experienced by persons with these disorders may predict the nature of the infections likely to be caused by use of a specific monoclonal antibody and vice versa.

12.3 Tumour Necrosis Factor Antagonists

Tumour necrosis factor (TNF), previously called cachectin and then tumour necrosis factor-alpha, is a systemic inflammatory cytokine able to induce apoptotic cell death, to induce inflammation, and to inhibit tumorigenesis and viral replication. It is produced mainly by macrophages, but also by a variety of other cell types including lymphoid cells, mast cells, endothelial cells, cardiac myocytes, adipose tissue, fibroblasts, and neuronal tissue. Large amounts of TNF are released in response to lipopolysaccharide, other bacterial products, and interleukin-1 (IL-1). TNF can bind to two distinct receptors, TNF-R1 (TNF receptor type 1; CD120a; p55/60) and TNF-R2 (TNF receptor type 2; CD120b; p75/80), TNF-R1 being found in most tissues but TNF-R2 only in cells of the immune system [4, 5]. Binding of TNF to receptors leads to activation of a complex array of inter-acting and often conflicting inflammatory responses including enhancement of activated B-cells, pathways involved in cell proliferation and both anti-apoptotic and pro-apoptotic responses. This necessitates a sophisticated signalling system and diverse factors such as cell type, concurrent stimulation of other cytokines, or the amount of reactive oxygen species can shift the balance in favour of one pathway or another. Such complicated signalling ensures that, whenever TNF is released, various cells with vastly diverse functions and conditions can all respond appropriately to inflammation [4, 5]. TNF appears to be important in augmentation of both the adaptive and innate immune systems.

There is no known human state of TNF deficiency, but a "knock-out" mouse model was first developed in 1997, with the genetically engineered mice lacking the gene to produce TNF [6]. TNF-deficient mice or those lacking TNF receptors have markedly increased susceptibility to mycobacterial infection [7] and also to other intra-cellular pathogens including Listeria [8], Salmonella, some viruses but also to some extra-cellular pathogens like *Streptococcus pneumoniae* [9]. Interestingly, in IRAK-4 deficiency in humans, a defect in Toll receptor signal transduction leads to diminished amounts of TNF and other pro-inflammatory cytokines, and patients are susceptible to infections with *S. pneumoniae* and Salmonella. To interfere with TNF in humans seems risky in the extreme, but the important role played by TNF in causing and prolonging inflammation in rheumatic and other auto-immune or inflammatory disorders led researchers to develop a number of different biologics which interfere with TNF. These are shown in Table 12.1.

Table 12.2 Biologics, their targets and their infectious risks

Target	Names of biologics	Technology	Indications	Infectious risks
Tumour necrosis factor	Adalimumab	Humanised Mab to TNF	Arthritis	Mycobacteria
	Etanercept	Receptor fusion protein	Psoriasis	Fungi
	Infliximab	Mab to TNF	Crohn's	Viruses
	Golimumab	Mab to TNF		PCP, listeria
B-cell	Rituximab	Mab to CD20	Transplant rejection, cancer, etc	PML, HepB reactivation
	Epratuzumab	Mab to CD22	Lupus	Unknown
T-cell activation	Abatacept	Fusion protein to CTLA4	Transplant rejection	Unknown
	Belatacept			PML, EBV-related proliferative disease
IL-6	Tocilizumab	Mab to IL-6	Arthritis	Slight increase, no specific organisms
IL-1	Anakinra	IL-1 receptor antagonist	Arthritis	Cellulitis, pneumonia
Complement	Ecalizumab	Mab to C5	PNH	Meningococcus

EBV Epstein-Barr virus, *IL* interleukin, *Mab* Monoclonal antibody, *PCP Pneumocystis carinii* (now *jirovecii*) pneumonia, *PML* Progressive multifocal leucoencephalopathy, *PNH* Paroxysmal nocturnal haemoglobinuria, *TNF* tumour necrosis factor

As predicted from the mouse model, use in humans of biologics that target TNF (adalimumab, infliximab) or TNF-receptor (etanercept) has been associated with increased susceptibility to mycobacterial infections, both tuberculosis and atypical mycobacterial infections [3, 10, 11]. Persons with latent TB are at high risk of reactivation, and the Product Information for all the TNF biologics in Table 12.1 states that patients should be screened for latent TB before commencing the biologic. A patient shown to have latent TB (positive tuberculin skin test and/or interferon-gamma release assay, normal physical examination and chest X-ray), should be commenced on chemoprophylaxis with one or more anti-tuberculous drugs. Monotherapy with isoniazid or rifampicin is usual, but it may be advisable to use two or three drugs, depending on risk factors for reactivation, notably the patient's degree of immunosuppression.

For TNF antagonists, as for other biologics, the exact degree of increased susceptibility is hard to quantify. In most of the analysed studies and comparisons, there were no significant differences in safety outcomes between adalimumab and control groups [3, 10]. Serious infections were significantly more frequent in adalimumab patients in only one study [11] with a RR (95 % CI) of 7.64 (1.02–57.18) and a NNH of 30.2. Similarly, based on 4 RCTs (1,231 patients treated with golimumab and 483 with placebo) no significant differences were noted between golimumab and placebo regarding serious adverse events, infections, serious infections, lung infections, tuberculosis, cancer, withdrawals due to adverse events and inefficacy and deaths [12].

On the other hand, there are increasing numbers of reports of serious infections associated with TNF antagonist biologics. These are primarily with intracellular pathogens: tuberculosis and atypical mycobacterial infections, fungal infections and viral infections. The fungal infections have been with Aspergillus, Candida and in the USA with histoplasmosis, coccidiomycosis and blastomycosis, and failure to recognise fungal infections early prompted an FDA warning [13]. Fungal infections are often disseminated rather than localised, have a high mortality (12 of 240 patients died who developed histoplasmosis while taking TNF antagonist biologics and were reported to the FDA) [3] (Table 12.2).

TNF antagonist biologics may be associated with reactivation of chronic hepatitis B infection, and with severe HSV and VZV infections,

including sometimes the macrophage activation syndrome [14].

The pattern of infections with TNF antagonist biologics resembles those of a patient with T-cell deficiency, and there are reports of patients taking infliximab and other TNF antagonist biologics developing *Pneumocystis jirovecii* pneumonia (PJP, PCP) a median of 8 weeks after commencing therapy [15]. In addition, there are individual case reports of patients on infliximab developing Listeria infections.

Patients with T-cell defects may also have increased susceptibility to extra-cellular pathogens and this appears to be the case for TNF biologics, in as much as there are reports of pneumonia, pyelonephritis, septic arthritis and septicaemia in association with their use in adult rheumatology patients [10, 11], although the biologics may be only one of multiple factors contributing to these infections.

12.4 B-cell Antagonists

There is increasing use of rituximab, a monoclonal antibody which targets CD20, a protein found mainly on B-cells, in the treatment of lymphomas, leukaemias, transplant rejection and some autoimmune disorders.

B-cell antagonists can cause hypogammaglobulinaemia with a risk of sinopulmonary infections. While initial reports of the use of rituximab were optimistic regarding safety, there are emerging concerns about reports of reactivation of hepatitis B [16], sometimes leading to fulminant disease, and of the development of progressive multifocal leukoencephalopathy (PML) due to JC virus [17].

The JC virus (JCV) is a human polyomavirus genetically similar to BK virus and SV40. It was discovered in 1971 and named after the two initials of a patient with progressive multifocal leukoencephalopathy (PML). The virus causes PML and other diseases only in persons with immunodeficiency, as in AIDS or during immunosuppressive treatment (e.g. organ transplant patients). The virus is very common in the general population, infecting 40–60 % of humans [18, 19]. Most people acquire JCV in childhood or adolescence. It is found in high concentrations in urban sewage worldwide, leading some researchers to suspect contaminated water as a typical route of infection. PML is a demyelinating disease, affecting the white matter, destroying oligodendrocytes and producing intranuclear inclusions. PML is similar to another demyelinating disease, multiple sclerosis, but progresses much more quickly. In a recent report of 57 cases of PML associated with rituximab, the mortality was 90 % [17].

Epratuzumab, which targets CD22 on human and malignant B-cells, has been used in lupus and in oncology patients. The preliminary data have not suggested any particular infectious risk, but increasing use may reveal a pattern similar to rituximab.

12.5 Interleukin-6 Antagonists

Interleukin-6 (IL-6) is both a pro-inflammatory and anti-inflammatory cytokine. It is secreted by T cells and macrophages to stimulate immune response to trauma, especially burns or other tissue damage leading to inflammation. In terms of host response to a foreign pathogen, IL-6 has been shown, in mice, to be required for resistance against *Streptococcus pneumoniae* [20].

The (IL-6) antagonist tocilizumab has been used mainly in adults with rheumatoid arthritis and currently has a benign safety profile. A Cochrane meta-analysis of eight randomised controlled trials (3,334 participants; 2,233 tocilizumab and 1,101 controls) found that the tocilizumab group was a non-significant 1.18 times more likely to have any infection or infestation [21]. However, a non-Cochrane systematic review of six studies found that the higher but usual dose of 8 mg/kg was associated with a significant increase in risk of infection (Odds Ratio of any infection = 1.30, of serious infection = 1.78) [22]. There was no clear pattern to the infections incurred by the tocilizumab group in either meta-analysis.
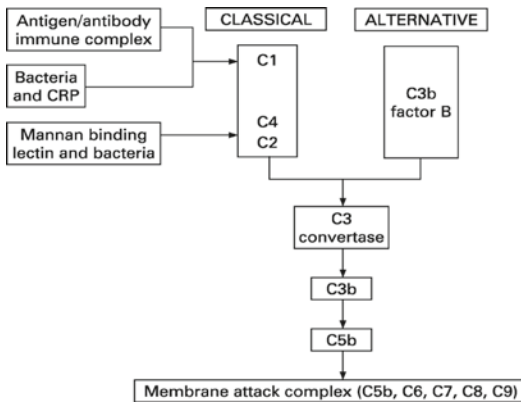
Fig. 12.1 Diagrammatic representation of the complement cascade

12.6 Interleukin-1 Antagonists

Interleukin-1 (IL-1) is produced in response to inflammatory stimuli including infection and mediates various physiological responses, including inflammatory and immunologic reactions, notably lymphocyte proliferation and fever. Anakinra (Kineret) is an IL-1 receptor antagonist, used in rheumatoid and sometimes in juvenile idiopathic arthritis. A Cochrane systematic review found 2,876 patients in trials, but the incidence of serious infections was only reported for 1,900 patients. The incidence of serious infections was "clinically higher", but not statistically different, in the anakinra (25/1,366 patients, 1.8 %) versus the placebo group (3/534 patients, 0.6 %). The pattern was mainly bacterial infections (cellulitis, bone and joint infections and pneumonia) [23].

12.7 Complement Pathway Antagonists

The monoclonal antibody eculizumab is being used increasingly to treat adult patients with paroxysmal nocturnal haemoglobinuria, whose red cells are susceptible to complement-mediated lysis. Eculizumab targets the C5 component of the complement cascade. We know that there are familial disorders that can cause deficiency of any of the terminal components or membrane attack complex of the complement cascade (C5 to C9).

The complement cascade occurs on the bacterial cell surface and the membrane attack complex punches a hole in the bacterial cell surface (see Fig. 12.1). Congenital deficiency in C5, C6, C7, C8 or C9 predisposes to meningococcal or gonococcal infections [24]. It is not surprising, therefore, that 3 of 196 adult patients receiving eculizumab developed meningococcal infection [25], and that meningococcal immunisation is strongly recommended for all patients starting eculizumab.

12.8 T-cell Activation Antagonists

Two drugs have been developed that block T-cell activation. Abatacept and belatacept are fusion proteins composed of an immunoglobulin linked to the extracellular domain of CTLA-4, which is a molecule crucial for T-cell co-stimulation, selectively blocking the process of T-cell activation. Abatacept and belatacept differ by only two amino acids. They are intended to provide extended graft survival. Preliminary studies have not reported significant infections with abatacept, but there have been concerning reports that belatacept has been associated with cases of PML and of EBV-related lymphoproliferative disease. The FDA has warned that concurrent use of abatacept and TNF antagonist appears to increase the risk of serious infection compared with TNF antagonists alone.

12.9 Diagnosis and Treatment

It is obviously critical to consider infections, including opportunist infections, in any patient who develops compatible symptoms when taking a biologic, and to follow the usual infectious disease practice of trying to obtain tissue to make a microbiological diagnosis.

12.10 Prevention

Appropriate preventative measures will depend on the nature of the biologic and the pattern of infections anticipated.

1. Screening for tuberculosis with Tuberculin Skin Test and/or interferon-gamma release assay: most relevant for TNF biologics, but also recommended for anakinra and tocilizumab.
2. *Pneumocystis* prophylaxis: consider with TNF biologics
3. Immunisation
   a. Meningococcal conjugate vaccine: all taking eculizumab, consider for B-cell biologics
   b. Pneumococcal conjugate vaccine: all taking biologics
   c. Hepatitis B: TNF biologics, B-cell antagonists (rituximab).

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Cat Scratch Disease and Other *Bartonella* Infections

13

Kenneth M. Zangwill

Abstract

First described in 1931, cat scratch disease remains the most commonly identified clinical syndrome associated with *Bartonella* infection. Over the last 20 years, however, the discovery and use of modern diagnostic tests has greatly expanded our understanding of the pathogenesis, clinical spectrum, and treatment options for *Bartonella* infections of all types. Indeed, each varies substantially depending on the infecting species and the immune status of the host.

13.1 Introduction

Bartonella species cause a wide range of clinical syndromes which vary substantially depending on the infecting species and immune status of the infected. Well known as a cause of trench fever and bartonellosis (a febrile illness endemic in the high valleys of the Andes mountains [1]), their recognition as human pathogens in the United States was virtually forgotten until the early 1990s when new diagnostic tests linked this genus to cat scratch disease. Today, the use of such tests has significantly broadened the clinical spectrum of cat scratch disease and also confirmed the role of *Bartonella* as the cause of bacillary angiomatosis (BA), an entity seen mainly among the immunocompromised.

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13.2 Making the Link Between *Bartonella* and Cat Scratch Disease

Cat scratch disease (CSD) was first described in a French boy seen in 1931 [2], but >50 years elapsed before evidence of its bacterial cause emerged. In 1983, Warthin-Starry staining revealed small bacilli among 34 of 39 lymph node samples from patients with CSD and in subcutaneous nodules from a patient with AIDS with unusual neovascular lesions subsequently recognized as BA [3, 4]. In 1988, it was proposed that CSD and BA may result from infection by the same organism based on the similarity of the morphologic and staining characteristics of these bacilli and that a history of cat scratches was often present in these patients. Importantly, antiserum raised against the "CSD bacillus" reacted with organisms in tissue samples from BA patients [5].

In 1990, molecular probe techniques (polymerase chain reaction (PCR)) suggested that BA

was caused by a bacterium similar to *Bartonella quintana* [6]. Other reports described morphologically similar-appearing organisms from patients with a vascular abnormality of the liver known as peliosis hepatitis [7] and febrile bacteremia [8], subsequently noted to be caused by both *B. quintana* and *B. henselae* [9–12]. Soon thereafter, a newly developed immunofluorescent antibody serologic test developed for BA was positive in 36 of 41 (88 %) serum samples from persons with suspected CSD, compared with only 6 % among healthy controls [13]; findings soon corroborated in a field trial among CSD patients in Connecticut, USA [14]. During this time, it was reported that CSD skin test antigen contained *Bartonella* nucleic acid sequences [15]. These observations confirmed the etiologic link between *Bartonella* and CSD and BA. Subsequently, organisms have been visualized microscopically or detected using PCR at the site of skin inoculation, lymph nodes, bone, eye, liver, and spleen in patients with CSD [16–21] and in nearly every organ, including the brain, among those with BA [22]. Regardless, the organism is only infrequently successfully cultured from clinical specimens among patients with either of these conditions.

13.3 Pathogenesis

Bartonella are facultative, intracellular Gram-negative bacilli with relatively fastidious growth characteristics. To maximize growth, tissue samples ideally should be directly plated onto solid media supplemented with hemin and incubated in a hypercapneic atmosphere. Most standard protocols for detection of such fastidious bacteria will allow for growth of *Bartonella*, but only after at least 7–10 days of incubation [23]. Currently at least 22 species have been described, 12 are known to cause disease in humans and the complete genome is available for 10 separate species.

In general, *Bartonella* establish infection within the reservoir host and eventually establish persistence within the erythrocyte. Several observations suggest that *Bartonella* may persist intracellularly: (1) persistent bacteremia has been noted in humans and a variety of mammals,

(2) isolation is enhanced by lysis centrifugation (which disrupts cellular membranes), (3) BA and trench fever patients may experience clinical relapse, and (4) better clinical response has been noted with antimicrobial agents that penetrate intracellularly. The defining pathogenic characteristics of human infection vary substantially with the infecting *Bartonella* species and also with immune status of the host. Transmission to humans can occur directly via an arthropod vector (e.g. sandflies and bartonellosis) or indirectly via inoculation (perhaps from infected flea feces) through intact skin via a cat scratch or other mucous membrane contact (CSD and BA). Many virulence factors involved in human infection have been identified that contribute to cellular invasion, erythrocyte persistence, pathologic angiogenesis, and evasion from host immune responses. A comprehensive review of *Bartonella* pathogenetic mechanisms has been recently published [24].

These primary clinical histopathologic manifestations range from focal granulomatous changes (CSD), multifocal angioproliferative changes (BA), endovascular multiplication (endocarditis), and an (proposed) exaggerated inflammatory response without evidence of bacterial invasion (meningoencephalitis). In CSD specifically, the lymph node histology reveals patchy, necrotic, granulomatous change with stellate abscesses and variable infiltration of leukocytes. This is in contrast to BA which typically reveals proliferation of small blood vessels with large, "plump" cuboidal endothelial cells with intermixed leukocyte infiltration and focal necrosis without granulomas, similar to that seen with bartonellosis.

13.4 Ecology

Various reservoirs for *Bartonella* species have been identified including feline, bovine, canine, cetacean, and rodent species. Most individual species, however, are relatively restricted in their distribution. Epidemiologic studies of patients with CSD and BA have shown that contact with cats, particularly kittens in the case of CSD, is associ-

ated with disease [14, 25]. Cats from households of such patients are more likely to have antibody against *B. henselae* than are control cats, seroprevalence of anti-*Bartonella* antibody among community cats can be very high (depending on the region) and overt feline bacteremia among community cats may exceed 50 % persisting for up to a year [14, 26–29]. Younger cats and stray cats are more likely to be infected with *Bartonella* than are older or domesticated cats [30].

It appears that each pathogenic *Bartonella* species may be transmitted by an arthropod vector, with variation by species. These vectors include flies, fleas, keds, lice, sandflies, and ticks, but most infections appear to be accidental in animal and human hosts. *B. quintana* and *B. bacilliformis* are transmitted by the human body louse and the sandfly, respectively. For *B. henselae,* the cat flea (*Ctenocephalides felis*) has been confirmed as a vector for CSD and/or BA through epidemiologic [14, 25] and experimental [14, 27, 31, 32] evidence. These data show that cat fleas can harbor *Bartonella* as detected by culture and PCR, cats bacteremic with *B. henselae* are more likely than nonbacteremic cats to be infested with fleas, and fleas can successfully transmit to *Bartonella* cats in a laboratory setting.

13.5   Cat Scratch Disease

Although definitive evidence is not available, it appears that the majority of infections caused by *Bartonella* species result in CSD, usually due to *B. henselae*. Large case series and systematic evaluations have defined the epidemiology of CSD. United States population-based surveillance data indicate that the incidence over all age groups is 3–4 per 100,000 population. Approximately 60 % of cases occur in persons <18 years of age with children 5–10 years of age at greatest risk (~10 per 100,000 population) [14, 33]. Familial and geographic clusters have been reported and incidence peaks in the winter months, with some geographic variability. Hospitalization for this illness occurs most commonly for surgical drainage or excision of affected lymph nodes with an annual incidence of ~0.6/100,000 children <18 years of age in the United States [34]. A case-

Table 13.1 Cat scratch disease: classic manifestations. (Data from [14, 38, 46, 66]

Clinical finding	Percent of patients
Lymphadenopathy	>90
Upper extremity	46–52
Neck/jaw	26–43
Groin	6–18
Suppuration	15–30
Fever	26–60
Inoculation site detectable	25–90
Malaise/fatigue/anorexia/ headache	10–45
Myalgia/arthropathy/tendinitis	3–11
Parinaud oculoglandular syndrome	5
Skin rash/erythema nodosum	3–5
Encephalopathy	<1

control evaluation noted that owning a kitten and being scratched, bitten, or licked by a kitten with fleas are the most important risk factors for disease [14]. Nearly 25 % of persons with CSD, however, do not report intimate contact with cats, though person-to-person transmission has not been documented.

Uncomplicated CSD is usually a self-limited illness characterized by lymphadenopathy that occurs after contact with a cat. A few days after a cat-scratch or bite, a papule or wheal frequently develops at the site which is followed by development of regional lymphadenopathy 1–2 weeks later. Concomitant constitutional symptoms occur in up to 60 % of cases including malaise, fever, sore throat, and/or headache (Table 13.1). Children <15 years of age are more likely to develop lymphadenopathy in the neck, whereas older persons are more likely to have enlarged nodes in the groin or axilla [33]. Up to one-third of patients will report lymphadenopathy at more than one site. Nodes generally are not tender, yet suppuration occurs in 15–30 % of cases with even deep neck space disease reported [35]. Prolonged bacteremia and even widespread dissemination involving multiple organ systems has been reported as well [36, 37]. In most cases of CSD, the clinical illness resolves spontaneously but lymphadenopathy may persist for weeks to months [38].

The widespread use of a serologic test against *Bartonella* species has rapidly and significantly expanded the clinical spectrum of this illness to include nearly every organ system. Delays in diagnosis are commonplace and occur most likely due to general lack of awareness with the potential for infection among patients who have a non-classic presentation. Central nervous system disease most commonly presents as encephalopathy with associated seizures in 40–50 % of patients [21]. Status epilepticus, cranial and peripheral nerve paresis, and intracranial masses also have been reported. Onset is usually 1–2 months after the development of lymphadenopathy, and complete and rapid recovery is the rule, but severe disease with sequelae may occur [21, 39, 40]. Cerebrospinal fluid analysis is usually normal, but lymphocytic pleocytosis is noted in approximately one-third of patients. Ophthalmic disease usually presents as Parinaud oculoglandular syndrome (bulbar conjunctivitis, preauricular lymphadenopathy, and conjunctival granuloma), but other manifestations are well-described and neuroretinitis in the form of a "macular star" is particularly distinctive [41, 42]. Hepatitis and/or splenitis is well-described as well, usually accompanied by prolonged fever, abdominal pain, arthralgias, and/or pleomorphic rashes. Back pain is a common presenting symptom in such children [43–45]. In addition, many patients have been reported with musculoskeletal disease (particularly in women >20 years of age) or osteomyelitis with vertebral disease and contiguous abscess formation being prominent [46, 47]. Pulmonary disease has also been described in which nearly all patients developed pleural effusion [48]. The majority of patients with non-classic disease recover completely, and no deaths have been directly attributable to CSD. Clinical disease appears to provide lifelong protection insofar as recurrent disease has been described very rarely [43].

13.6 Bacillary Angiomatosis

BA is an uncommon manifestation of *B. henselae* or *B. quintana* infection seen primarily in adults with acquired immunodeficiency, usually during the late stages of AIDS [22]. The incidence and seasonal distribution of BA is unknown. Being scratched or bitten by a cat has been established as a risk factor for BA, but overall, one-third of patients did not report such contact in one study [25]. The clinical manifestations of BA are quite varied and may involve nearly every organ system but cutaneous, osseous, hepatic (peliosis hepatis), and splenic disease are the most commonly reported [22]. The range of cutaneous disease is broad, usually including superficial, erythematous, highly vascular, exophytic lesions or subcutaneous nodules. Lesions occur singly, or hundreds of lesions may occur in the same patient. Bone disease usually is painful and localized to tubular bones, with osteolysis noted on roentgenogram. Hepatosplenic disease often presents with prolonged fever, abdominal pain, and substantial weight loss. Only a few instances of BA have been reported among children some of whom were immunocompetent [49–51].

13.7 Other Syndromes

In addition to "classic" CSD and BA, *Bartonella* species have been linked to several other syndromes. *B. quintana* and *B. henselae* bacteremia, most commonly seen among the homeless or those infested with scabies or lice, may persist for prolonged periods despite antimicrobial therapy [8, 11, 12, 52]. Endocarditis caused by several *Bartonella* species has been described and now should be included as a potential cause of "culture-negative" HACEK-type endocarditis, i.e., endocarditis caused by fastidious Gram-negative organisms. Such patients usually have one or more underlying conditions, including infection with HIV, chronic alcoholism, or homelessness [53–56]. The homelessness and infestations associated with *Bartonella* infection noted above parallel conditions experienced by soldiers with trench fever in which prolonged bacteremia with *B. quintana* was also reported.

Lastly, *Bartonella* have been implicated in several different central nervous system syndromes which may represent rare forms of CSD or BA, or unique manifestations of *Bartonella* infection altogether. These syndromes, most frequently noted in patients with HIV infection,

include aseptic meningitis, transient cranial and/ or peripheral nerve dysfunction, aphasia, alteration in mood or affect, psychoses, space-occupying lesions, and an acute psychiatric complex [41, 57, 58].

13.8 Diagnosis

The approach to diagnosis of infection with *Bartonella* varies with the clinical presentation. For classic CSD, particularly in immunocompetent hosts, the diagnosis can usually be confidently made with the subacute emergence of regional lymphadenopathy with or without constitutional symptoms in persons with prior traumatic contact with a cat or kitten distal to the affected node. In such instances, serologic testing, molecular diagnostics, or imaging is usually unnecessary.

Other *Bartonella* syndromes, however, require the use of serologic testing or clinical specimens for microbiologic and/or molecular evaluations. Currently, serologic testing is the mainstay of diagnosis and is most commonly done using a commercially available enzyme immunoassay or an indirect immunofluorescence assay for *Bartonella* IgM and IgG antibody levels. The sensitivity of these tests is variable and may be low, but specificity is generally quite high [59, 60]. Among patients with CSD, antibody titers peak at 4–5 months after onset of symptoms and have been shown to persist for up to 3 years [14, 61]. Serologic tests have completely supplanted the use of the CSD skin test, a non-standardized preparation previously available in limited quantities, originally developed in 1946 [2]. Currently available serologic tests are not able, however, to differentiate reliably between species of *Bartonella*. Molecular techniques are also available in many commercial laboratories, but the methods are not standardized.

Bartonella species are relatively fastidious and recovery is maximized with the use of lysis centrifugation technique and selective media that contain hemin, followed by growth in a hypercapnic atmosphere for at least 10 days. Tissue samples may reveal *Bartonella* with the use of a range of silver stains and Gram's stain is generally insensitive for this pathogen.

13.9 Therapy

The majority of mild-to-moderate cases of CSD resolve in 1–2 months without any antimicrobial therapy. Although *Bartonella* species appear to be susceptible to several antimicrobials when tested *in vitro*, such results do not predict reliably the clinical response to therapy; use of such tests as a clinical tool should therefore be discouraged.

Only one prospective controlled study of therapy for cat scratch disease has been published [62]. This study reported on 29 patients who were randomized to azithromycin (for 5 days) or placebo after diagnosis was confirmed by serologic testing. The outcome measure was change in the (largest) lymph node size, as determined by ultrasound 30 days after initiation of treatment. No other clinical signs or symptoms were reported and drug compliance was not monitored. The authors note that those in the treatment group had significant improvement in nodal size and suggested that azithromycin be considered for therapy of this condition.

This study, however, was flawed in several important areas rendering its conclusions highly suspect [63]. There were only 14–15 patients per group, >50 % of whom in each group received at least one antimicrobial before randomization and the details of type and duration of antimicrobial use are not presented. Very little information was presented with regard to clinical resolution of signs and symptoms (aside from the ultrasound data) and interestingly there was little correlation between physical examination and findings on ultrasonography of lymph nodes. As well, the authors report a statistically significant difference in nodal size at 30 d, but this difference was not present by 60 d. The clinical relevance of the ultrasonographic findings, specifically for deciding about the need for antimicrobial therapy in the absence of demonstrable clinical benefit, is unclear. Anecdotal evidence and retrospective series suggest that some clinical response may be achieved for patients with CSD with trimethoprim-sulfamethoxazole, rifampin, gentamicin, and in adults, ciprofloxacin.

A combination of therapeutic restraint, pain control, and reassurance remains the most

prudent approach to the great majority of patients with uncomplicated CSD in whom clinical symptoms and signs, as well as lymphadenopathy, will likely spontaneously resolve over several weeks to months. Antimicrobial therapy may be considered for those patients (1) in whom lymphadenopathy does not resolve over a reasonable period, (2) in whom lymphadenopathy is associated with significant morbidity, such as pain or persistence of debilitating constitutional symptoms, (3) with severe systemic disease; i.e., encephalopathy, osteomyelitis, or neuroretinitis, and/or (4) with an underlying medical disorder complicated by severe CSD. Specific recommendations for these circumstances cannot be definitive as controlled data are unable, but an expert panel has published guidelines [64].

Aspiration of inflamed lymph nodes only should be performed to rule out more serious diagnoses or if a treatable superinfection is suspected. In such cases, either a fine-needle aspiration or excisional biopsy should be done; incision and drainage procedures may promote the development of fistulae. No specific measures regarding antimicrobial prophylaxis of close contacts of the implicated cat itself can be recommended. Also, declawing of the cat or its removal from the household is not necessary.

Patients with BA usually respond to macrolides or tetracyclines. Uncomplicated cutaneous disease in HIV-infected patients requires at least 6–8 weeks of therapy, whereas more severe or invasive disease may require months of therapy or perhaps lifetime suppressive therapy. Others have proposed specific treatment regimens for BA, bacteremia, and endocarditis caused by *Bartonella* that include various combinations of gentamicin, erythromycin, or ceftriaxone [64].

13.10 Opportunities

With the wide availability of serologic and molecular diagnostic tools, new information about *Bartonella* infections will likely continue to emerge. Important knowledge gaps remain in nearly all domains of this organism as a human pathogen including the dynamics of its ecology and transmission in nature, human pathogenesis, relationship of the host to the pathogen, and differing responses to antimicrobial therapy. Cats remain the most important reservoir for CSD and BA—and over one-third of all U.S. households include at least one cat [65]—perhaps new information will lead to a preventive approach such as vaccination of cats.

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When to Think of Immunodeficiency?

14

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Abstract

Primary Immunodeficiencies (PIDs), although rare, are serious and heightened clinical suspicion leads to earlier diagnosis and improved outcome. Recognition of PIDs may be difficult as infections are common in young children in particular. Clues to the diagnosis of PID may be found in history, examination and initial basic investigations such as lymphocyte count. Age at presentation, type of infective organism and family history help focus on likely PIDs. Type of infective organism may indicate a specific PID, for example Aspergillus and Chronic Granulomatous Disease and Pneumocystis Jiroveci and SCID amongst others. Diagnostic aids such as 'The 10 Warning Signs of Primary Immunodeficiency' can be useful with failure to thrive, need for IV antibiotics, and family history of severe or unusual infections being the most discriminating. Systemic examination including the recognition of dysmorphic features may also support a particular diagnosis.

14.1 Introduction

Infections are common during childhood and young children in particular are often exposed to new pathogens. Responding to recurrent infection is also part of establishing a mature and functional immune system. The paediatrician must decide when the pattern and type of infection is not normal but indicates that a child could have an underlying immune defect so as to strike a balance between over-investigating every child who sneezes whilst not missing a potential immunodeficiency.

Primary Immunodeficiency (PID) is not much less common than conditions such as coeliac disease and cystic fibrosis yet presents with non-specific signs. Pattern recognition and awareness of the important warning features are key to early investigation and/or referral [1].

The importance of diagnosis cannot be understated, as earlier treatment can prevent end organ

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damage and ensure that treatment, be it curative, such as Stem Cell Transplantation, or supportive, such as immunoglobulin, will enable the patient to lead a healthy normal life. Diagnosis also has implications for the wider family as it should facilitate genetic counselling, so identifying other family members at risk of the same condition.

14.2 What is Compromised?

Defects can be classified according to the aspect of the immune system which is affected. Slightly modifying The International Union of Immunological Societies classification, Primary Immunodeficiency can be divided into six categories [2]:

- Combined T and B cell immunodeficiencies
- Predominantly Antibody Deficiencies
- Defects in Innate Immunity, including phagocytic cell and complement defects
- Other well-defined Immunodeficiency Syndromes
- Diseases of Immune Dysregulation
- Autoinflammatory Disorders

The presentation of these conditions differs depending upon which component of the immue system is compromised; however, there are overlapping features. The first step is not to diagnose the specific defect but to be aware of what may be compromised, how it may present and what investigations should be performed. As an approach to PID, we suggest considering the following:

- Age
- Organisms
- Systems involved
- Family history
- Haematology

These components shall now be considered.

14.3 Age

The immune system develops and matures as the child grows older, during which time, the child is exposed to many microbes transmitted by parents, siblings, other children at daycare and the wider environment. It is therefore, unsurprising that infections are frequent in young children, but the more serious the defect the more quickly a child with PID becomes ill, so age can itself be a clue to the underlying diagnosis and its severity.

14.3.1 Less than 6 months

As the inuterine is sterile, PID is not embryologically fatal. However, once born, infants are exposed to a vast array of potential organisms. They are immunologically naive and are reliant on the innate and primary adaptive responses, as well as protection from transplacentally-acquired maternal antibody.

The more severe the condition, the earlier the presentation. A child with absent T and B cell function will suffer serious consequences from even a usually trivial infection such as Rotavirus enteritis. Presentation so early in life is highly suggestive of T cell deficiency or defect, and these infants often present with failure to thrive and/or severe infection. They are likely to have a severe underlying diagnosis such as severe combined immunodeficiency (SCID), T lymphocyte disorders, DiGeorge, Wiskott Aldrich syndrome and chronic mucocutaneous candidiasis.

Delayed separation of the umbilical cord beyond 14 days is very suggestive of Leukocyte Adhesion Deficiency (LAD), whilst a pronounced milial rash on the face may indicate Hyper IgE syndrome. Severe congenital neutropenia also presents at this time with severe bacterial infection.

Non-immunological manifestations of syndromes such as the cardiac features associated with DiGeorge may also present and prompt the clinician to look for a PID.

14.3.2 6 months to 5 years

By the age of 6 months maternal antibodies have waned and the nadir of their protection reached. A lack of antibody production or function due to conditions such as X linked agammaglobulinaemia, CD40 Ligand deficiency or one of the rare autosomally recessively inherited causes of

Table 14.1 Organism and disease

Organism	Disease to consider
Burkholderia cepacia	CGD
Pneumocystis jiroveci	SCID, CD40 L, ICF
Aspergillus	CGD, Hyper IgE
Candida	Chronic mucocutaneous candidiasis
Haemophilius	Specific antibody failure
Meningococcus	Complement deficiency
Pneumococcus	Asplenia
Staphylococcus aureus	CGD, Hyper IgE

Table 14.2 Causes of *Pneumocystis jiroveci* pneumonia

Malignancy and immunosuppression
HIV
SCID
CD40 ligand
MHC II
ICF
NEMO
Hyper IgE

antibody deficiency becomes apparent after 6 months of age. Forty two percent of patients with an antibody deficiency have presented within the first year of life, whilst 88 % have presented by the age of 3 years [3]. 'Phagocyte disorders' including chronic granulomatous disease as well as the various forms of Haemophagocytic Lymphohistiocytosis such as Chediak Hegashi, also present at this age.

It is also possible there may be late presentations of SCID and this diagnosis should not be excluded on age alone.

14.3.3   5 years and Older

By 5 years old, the immune system has developed and matured considerably. There has been a decline in reliance on the innate immune system, whilst adaptive immunity has developed more fully. Children over the age of 5 years are most likely to have diagnoses such as Common Variable Immunodeficiency, specific antibody deficiency and complement disorders.

14.4   Microbial Clues

Whilst no organism is truly pathogneumonic of a PID, there are certain organisms which when present should immediately trigger thoughts of immune dysfunction. Table 14.1 highlights some of the organisms which are particularly associated with specific conditions, some of which will now be discussed in more detail.

14.4.1   Fungal Clues

Any invasive fungal infection is unusual in a child with a well functioning immune system and must always be considered as a 'red flag'.

Pneumocystis jiroveci (*Pneumocystis carinii*) is one such 'red flag' organism. Pneumocystis is virtually unheard of in the immunocompetent host. It is the most likely opportunistic infection in SCID but its presence should also alert the clinician to one of the underlying diagnoses shown in Table 14.2. It occurs most commonly in the 3–6 month age group and it may result in the need for significant respiratory support including artificial ventilation.

Patients with Chronic Granulomatous Disease (CGD) have defective oxygen-independent killing mechanisms and are particularly susceptible to fungal infection. Their infections are indolent and recurrent in nature. They are particularly susceptible to members of the *Aspergillus* genus and the presence of this organism necessitates excluding a diagnosis of CGD. *Aspergillus nidulans* seems to have a particular predilection for CGD and it almost never causes disease in other immunocompromised patients such as those with febrile neutropenia due to chemotherapy [4].

Candida is part of the normal flora of healthy individuals and infants may be colonised from early life. In individuals with well functioning immune systems it rarely causes significant disease. However, as a manifestation of PID it is significant and in particular, it may point to a diagnosis within the spectrum of conditions of chronic mucocutaneous candidiasis [5] or as part

of SCID. Invasive candidial infections or persistent candida should be considered part of the spectrum of PID and warrant investigation.

14.4.2 Bacterial Clues

The pneumococcus is a challenging organism for even the immunocompetent child. The polysaccharide capsule ensures that the organism is difficult to opsonise and optimal antibody function is imperative to successfully defeat the organism. These features make it particularly relevant in patients with asplenia.

Meningococcal is known to thrive when there is a deficiency in the complement pathway. The incidence of complement deficiency varies greatly from one population to another; in the Israeli population the incidence is three times that of other countries included in the ESID registry with 16.3 % of PID in Israel due to complement deficiency [6]. However, in England and Wales, where there are approximately 1,000 cases of meningococcal disease occurring per year [7], a review of 297 cases of meningococcal septicaemia and meningitis found only one case of complement deficiency and of note this case had a previous serious infection [8]. Investigation for PID in those who have had meningococcal disease should be reserved for:
- Recurrent meningococcal disease
- Serotype W135 or Y
- Previous bacterial infection
- Positive family history (of PID or severe or recurrent meningococcal disease).

The experience with meningococcal disease highlights that the presence of invasive bacterial infection whilst important is not diagnostic of PID.

Staphylococcus aureus is a relatively common organism. However, it is well recognised as being a common presenting organism in CGD. Suppurative adenitis was present in 40 % of cases of CGD in a UK registry, and was the commonest presenting feature. When an organism was isolated it was always *S. aureus* [9]. This bacterium was also the commonest organism in liver abscesses in CGD patients [9].

Failure to properly handle mycobacteria may present further information about immune function. The United Kingdom is now one of several countries who have adopted a targeted neonatal BCG programme [10] rather than universal immunisation. This may be crucial in the presentation of PID. A localised reaction at the site of the BCG and the involvement of a single lymph node may be normal. Disseminated BCG infection however, is not seen in the immunocompetent individual and should be considered a manifestation of PID until proven otherwise [11]. Increased susceptibility to mycobacteria is seen in disorders of phagocytic function. Individuals with SCID, CGD and those with disorders of the IFN-γ/IL-12 pathway are particularly susceptible to severe disease [12].

14.4.3 Viral Clues

Viruses associated with a particular system will be discussed later in this chapter. At this point it is worth considering how failure to deal appropriately with viruses may be a significant presentation of PID.

The human herpes viruses are an example of a group which have a profound effect on the immunodeficient child. A considerable amount is known about the normal course of infection with various members of the herpes family.

Varicella zoster virus (VZV) infection remains common in childhood in countries where universal vaccination has not been implemented. In the majority of children, it is a mild illness requiring symptomatic treatment only, serious complications are relatively rare with bacterial sepsis being the most common. [13] However, in the child with an underlying PID, particularly a T cell defect even this typical childhood illness can be life threatening. In particular children with Cartilage Hair Hypoplasia are at risk of Varicella Pneumonitis. A family member who has died from VZV should also be considered an unusual consequence of this childhood illness.

Epstein Barr virus (EBV) infects nearly all children and patients are either asymptomatic or suffer an infectious-mononucleosis illness which is self limiting. Overwhelming EBV infections causing Haemophagocytic Lymphohistiocytosis is a common presentation of X-linked

proliferative disease; hypogammaglobulinaemia, lymphoma, aplastic anaemia and vasculitis being other ones. Other PIDs such as ITK deficiency may present as atypical lymphoma, such as extra-nodal Hodgkin's disease involving the lungs, induced by EBV.

14.5 Systems

14.5.1 Respiratory Clues

Respiratory infection is almost universal in childhood. Furthermore the lungs are vulnerable to infection as they have to compromise the immune defence capability to fulfil their gas exchange function. The healthy child has numerous respiratory tract infections a year with a significant number occurring in winter. Additional risk factors such as passive smoking, day-care attendance or atopy put specific cohorts of children at further risk. It is therefore, all too easy to dismiss the child with an immune deficiency who presents with respiratory distress. There are, however, red flags within the respiratory illnesses to alert us to the children who are not run of the mill.

Common conditions, taking uncommon courses should prompt the clinician to consider a diagnosis of PID. For example, respiratory syncytial virus (RSV) has a very predictable course, with the majority of children improving over 7–10 days and with a low mortality rate [14]. Those children who have chronic RSV or persistent bronchiolitis need to be considered to have an immune defect and of those who do, SCID is top of the differential.

Pneumatocoeles (as shown in Fig. 14.1) are unusual in childhood infection and their occurrence should alert the clinician to an unusual diagnosis at the very least. They are typically associated with *S. aureus* and subsequently with Hyper IgE Syndrome. Their presence should be taken to assume a diagnosis of Hyper IgE syndrome until proven otherwise.

Recurrent infection at the same site should always alert the physician that all is not well. It may be that there is an anatomical anomaly which accounts for the recurrent infection, for example an undiagnosed tracho-oesophageal fistula. How-

Fig. 14.1 Pneumatocoeles on chest X-ray. (Source: The Paediatric Immunology Unit, Great North Children's Hospital, Newcastle upon Tyne, NE1 4LP)

ever, it may be that it is the immune system itself, leaving that particular site susceptible to infection. In the case of recurrent pneumonia, several immune deficiencies may present in this manner, namely, XLA, common variable immune deficiency (CVID) and antibody deficiencies.

The commonest cause of bronchiectasis in the UK currently, is Cystic Fibrosis (CF). However, through the introduction of newborn screening, it is hoped that many cases will now be picked up shortly after birth [15]. It is therefore, even more imperative that when bronchiectasis is present, an underlying immune deficiency is considered. The most likely cause is an antibody deficiency. Ideally, this would have been diagnosed prior to the development of bronchiectasis, but even once bronchiectasis is evident the earlier this diagnosis is made the better as antibody deficient patients with established bronchiectasis at the time of diagnosis have a poorer prognosis than those who do not.

14.5.2 Gastroenterological Clues

The gastrointestinal tract requires a huge permeable surface area if it is to effectively digest and absorb nutrients. This renders it vulnerable to exploitation by microbes and so a multitude

of defence mechanisms have evolved to protect against infection. These comprise both non-specific innate immune responses as well as specific adaptive responses. It is also colonised by bacteria constituting the normal flora which in the immunocompetent host serve to stimulate protective non inflammatory responses rather than pathogenic ones.

Gastrointestinal (GI) symptoms, especially diarrhoea and vomiting are common in young children and repeated exposure to viral pathogens in particular induce protective responses that in time prevent infection. Gut disease as a manifestation of PID is often misdiagnosed as a primary GI problem. Whilst most cases of diarrhoea and vomiting are caused by uncomplicated viral infection in an immunocompetant child, this may be the first presentation of SCID, HIV or a T cell activation defect. Failure to clear an enteric virus such as adenovirus, rotavirus, norovirus or enterovirus should trigger investigation for a PID, especially when associated with chronic failure to thrive, which in infancy may be the first presentation of a severe PID and in particular SCID or HIV.

Chronic diarrhoea with or without the passing of blood or mucous is seen in 15 % of CGD patients. These important symptoms are indistinguishable from those seen in Crohn's Disease. However, there are important differences in management. If CGD is not diagnosed and immunosuppression given without antibiotic and antifungal prophylaxis, severe disseminated infection with organisms such as *Burkholderia cepacia* can ensue.

Hepatic manifestations of PID have been regularly described [16, 17] and these range from abnormal liver enzymes to sclerosing cholangitis (SC) [18]. The most common conditions associated with hepatic involvement are CD-40 ligand deficiency and X-linked Hyper-IgM syndrome. There is a high association of infection with *Cryptosporidum parvum* and the development of liver disease in patients with PID [18] and the co-existence of these two conditions should raise the possibility of PID as a unifying diagnosis. Sclerosing cholangitis is uncommon in the paediatric population, but it is not uncommon in Hyper-IgM syndrome [19] where liver involvement, including SC, occurs in as many as 75 % of patients [17], or CD40-ligand deficiency where SC can be present in around one third of patients [20].

Liver abscesses are an uncommon presentation in the paediatric setting in the developed world. Recurrent, persistent and multiple hepatic abscesses are the hallmark of CGD with *S. aureus* being the most common causative organism [21]. Diagnosis of a hepatic abscess in a child should be considered the starting point of investigation and not the end point. An underlying PID should be sought and in particular CGD must be considered. CGD is X-linked in 70 % of cases [22] but both X-linked and autosomal disease may present in this manner and therefore, gender does not discriminate in this diagnosis.

14.5.3 Skin

The skin presents the first part of the immune system; a mechanical barrier to pathogen entry, vital in the defence of the body from infection. It is, therefore, unsurprising, that children with defective immune systems may present with dermatological manifestations.

Eczema is common throughout childhood and it has several associations with PID. It may be a presenting feature in Wiskott Aldrich syndrome (WAS), Hyper IgE Syndrome (Job's), DOCK8 mutation and SCID.

In WAS, eczema is one of the defining features. However, it may be mild and there are often, small petichial haemorrhages and associated bleeding or bruising. This is in contrast to the findings in SCID. In patients with Omenn syndrome, the skin is thick and leathery with associated rubbery, large axillary and inguinal lymph nodes. Figure. 14.2 shows the classic appearance of Omenn's skin. In patients with SCID with MFE the rash is similar to that in Omenn but may well be milder. DOCK8 features extensive severe eczema with food allergy but in a significant proportion of cases this is accompanied by extremely extensive Molluscum contagiosum (as shown in Fig. 14.3) and/or human papilloma viral warts which often become malignant.
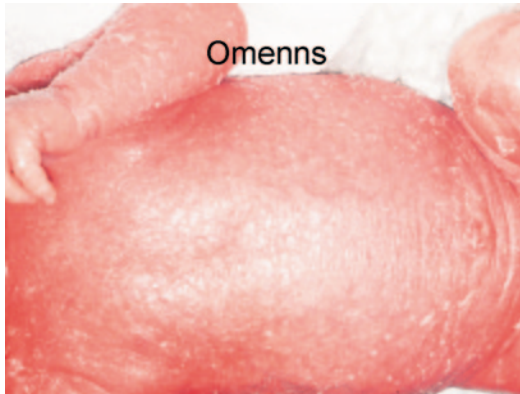
Fig. 14.2 Omenn's skin. (Source: The Paediatric Immunology Unit, Great North Children's Hospital, Newcastle upon Tyne, NE1 4LP)

Fig. 14.3 Extensive Molluscum contagiosum. (Source: The Paediatric Immunology Unit, Great North Children's Hospital, Newcastle upon Tyne, NE1 4LP)

Persistent or recurrent skin or mucosal infections, in particular with candida, may be the presentation of SCID, chronic mucocutaneous candidiasis, Autoimmune Polyendocrinopathy-Candidiasis-Ectodermal Dystrophy (APECED) or Hyper-IgE Syndrome. In SCID, there is often superficial candida, in particular in the mouth and napkin area, often with the presence of satellite lesions. Invasive candidiasis, however, is not a feature. In APECED, the presentation of candida is very variable. However, the candida when present may be difficult to treat, with topical treatments offering no relief. Systemic and repeated courses of fluconazole may be required. Associated nail dystrophy, dental enamel hypoplasia and autoimmune disease are pivotal in the diagnosis of this syndrome.

14.5.4 Other Features

Paediatricians are trained to recognise patterns of syndromic features. Whilst some facial appearances are striking and proclaim the diagnosis immediately, others are more subtle and must be sought alongside examination of other family members. These fundamental observations are as much a part of PID as any other speciality in paediatrics.

The classic DiGeorge facies are not always immediately recognised but once realised to be present should prompt examination of T cell number and function and look for 22q11 deletion. The classic DiGeorge facies include a button nose, underdeveloped philtrum and small ears [23].

The asymmetrical face with a prominent forehead and deep set eyes, hypertelorism, and cathedral palate [24], alongside the recurrent staphylococcal infection and red, itchy skin should prompt the diagnosis of Hyper IgE syndrome. Even when dysmorphic features are noted it may not always be possible to link them to a diagnosis, but nevertheless, their presence or absence should be sought and noted.

A thorough examination and history of dentition is required. Both a lack of teeth or a persistence of baby teeth may be relevant and if not specifically considered may be easily forgotten. The persistence of baby teeth may appear innocuous but may be part of Hyper IgE Syndrome (Fig. 14.4). The lack of teeth or the presence of conical shaped incisors (Fig. 14.5) should raise suspicion of a defect in the NEMO pathway.

Examination of the mouth by either paediatrician or dentist can yield crucial information. The presence of severe gingivitis may indicate CGD, CVID, Chediak-Higashi, Wiskott-Aldrich or Kostmann Syndrome. Recurrent aphthous ulcers, may often be thought of in connection with GI diseases such as Crohn's but may also be the hallmark of CGD, cyclical neutropenia and Hyper IgM or IgE disease. Oral candidasis beyond infancy can be the result of chronic

Fig. 14.4 Typical IgE teeth. (Source: The Paediatric Immunology Unit, Great North Children's Hospital, Newcastle upon Tyne, NE1 4LP)

Fig. 14.5 The conical incisors seen in NEMO defects. (Source: The Paediatric Immunology Unit, Great North Children's Hospital, Newcastle upon Tyne, NE1 4LP)

mucocutaneous candidasis and hyper IgE syndrome. Periodontitis is characteristic of many PIDs including LAD, Wiskott-Aldrich, Chediak-Higashi and selective IgA deficiency. The eyes may be the window to your soul but the oral cavity may offer an insight into the functionality of your immune system. [25]

Partial oculocutaneous albinism is an unusual presentation worldwide and the detection of it should prompt consideration of Chediak-Higashi as this feature is well described [26, 27].

14.6 Family History

A family history of PID is a strong clue to a potential diagnosis of PID and is considered one of the three most important warning signs for a potential diagnosis of PID, alongside the need for IV antibiotics and failure to thrive [28]. However, the family history may not be known to the wider family or its importance not appreciated or understood. Therefore, more specific questions need to be asked to elicit relevant family history, and it is often worth asking about family history of disease on more than one occasion as more than one prompt may be needed for a relevant history to be revealed. Consanguinity needs to be specifically asked about in all ethnic groups

as the prevalence of autosomal recessive PID is significantly higher in this group.

Questions should include,

- unexplained death in infancy within the family
- a history of recurrent or severe infections in a family member
- a history of abscesses particularly requiring surgical intervention or recurrent in nature
- the presence of malignancy in family members, e.g. lymphoma in male relatives may indicate XLP or Wiskott-Aldrich syndrome
- autoimmunity
- a history of eczema and bleeding.

Death from infection in infancy must raise the possibility of a diagnosis of SCID. This is particularly pertinent in the male relatives due to the prevalence of X-linked conditions. Autoimmunity is seen in female carriers of certain conditions, for example, in CGD the female carriers have a high incidence of SLE [29]. Autoimmunity should not be dismissed as unconnected. As our understanding develops we are aware of more associations that may be relevant.

14.7 Haematological Clues

Contrary to popular perception diagnosing PID does not start with complicated investigations even though they may be part of the diagnostic

process. A simple full blood count (FBC) can yield crucial information and guide the physician to further appropriate investigations. Each component of the FBC should be scrutinised.

White cell count: Whilst the total white cell count is usually normal even in SCID, a closer look at the differential will often reveal a lymphocyte count $<2\times10^9$/litre. It is often forgotten that whilst a total lymphocyte count above 1.0×10^9/litre is normal in older children and adults, in infancy the lower limit of normal is between 2.5 and 2.7×10^9/litre. Thus in any infant with an infection, a lymphocyte count below this on two occasions should lead to consideration of a diagnosis of SCID [30]. A neutrophil count of $<1.5\times10^9$/litre should cause alarm bells to ring and the possibility of cyclical neutropenia and CD40 Ligand deficiency considered.

Platelets: Thrombocytopenia is seen in WAS, and whilst counts of $70–80\times10^9$/litre are often seen in young infants with WAS, they fall with age. The platelets themselves are classically small with the mean platelet volume typically being half that of healthy controls (3.8–5.0 fl) [31]. It is imperative, however, that the Coulter counter is calibrated prior to testing in order that accurate results are given. Thrombocytopenia may also be part of a pancytopenic picture.

Red cells: The presence of erythrophagocytosis seen on a blood film is unusual and may be due to XLP. Aplastic anaemia may also be associated with XLP and this would be demonstrated on the blood film.

14.8 Overall Impression

Throughout this review, individual factors which that could point to a potential PID have been highlighted. They are outlined to prompt thought about an underlying diagnosis but as with all clinical medicine should not be seen in isolation but rather should prompt the physician to re-visit relevant aspects of the history and physical examination

with a new insight. Overall impression, or 'gut instinct' may also alert the paediatrician and this of course should not be ignored.

14.9 Who to Investigate?

Throughout this review, there has been a focus on common presentations of PID in childhood. The National Institute for Health drew attention to ten warning signs which indicate PID [32] to further educate physicians about immune deficiency and they are:

- Eight or more new ear infections within 1 year
- Two or more serious infections within 1 year
- Two or more months on antibiotics with little effect
- Two or more episodes of pneumonia within 1 year
- Failure of an infant to gain weight or grow normally (failure to thrive)
- Recurrent deep skin or organ abscesses
- Need for intravenous antibiotics to clear infections
- Two or more deep seated infections e.g. sepsis, meningitis
- A family history of PID
- Persistent thrush in mouth or fungal infection on skin.

However, when evaluated further, the three most critical of these are:

- Failure of an infant to gain weight or grow normally (failure to thrive)
- Need for intravenous antibiotics to clear infection
- Family history of PID [28].

14.10 How to Investigate?

It is not possible in this review to describe the full range of possible investigations for PID and so it shall focus on some core and first line investigations.

The importance of the total lymphocyte count in the diagnosis of SCID has already been highlighted. To further investigate, it is necessary to look at the numbers of T and B lymphocytes and

NK cells. Infants with significant T cell defects will have reduced T cell numbers in all subsets, but may have raised, low or normal B cell and NK cells. Lack of naïve T lymphocytes characterises unusual forms of SCID, and the expression of common gamma chain is defect in this form of X linked SCID, so these and other markers can be helpful in delineating the exact type of SCID. Further investigation into T cell function may be required. This can be determined by measurement of lymphocyte proliferation *in vitro* to mitogenic stimulation. In infants with severe T cell defects there will usually be very low (<10 % of normal) or no response to stimulation. Genetic studies pinpointing the exact gene defect are increasingly useful.

Measurement of serum immunoglobulins is an important part of the diagnostic process for PID. IgG, IgM and IgA should all be measured along with IgG subclassses. Age-specific normal values must be referred to when appraising these results. In young infants, the IgG present may represent transplacentally-acquired antibody. Immunoglobulin measurement is an important part of assessment but the results may be misleading with values not infrequently borderline in infants with well functioning immune systems and immunoglobulin present in low levels in those with SCID. Patients with B cell defects have low levels of immunoglobulins and some have low or absent numbers of B cells. In some antibody deficiencies B cells are present, but the lack of class-switched memory B cells makes a significant antibody deficiency likely.

Further information can be gleaned by the measurement of response to vaccinations. In the child that is already at least partially immunised, measurement of antibody response to tetanus and *Haemophilus influenzae* type b is critical. When measuring response to pneumococcal vaccination serotype specific response should be sought. When vaccinations have not been performed then they can be given and serological responses measured four weeks after.

The diagnostic test for CGD is flow cytometric assay of oxidative metabolism using dihydrorhodamine. The Nitroblue Tetrazolium (NBT) reduction test may also be used in the diagnosis, but the results are more subjective. It is worth noting that it is a *negative* NBT test that confirms the diagnosis of CGD!

When investigating for defects in the complement pathway it is critical that the investigations are not limited to C3 and C4 which may be considered laboratory standard. A more comprehensive assessment for complement deficiencies involves measuring both classical and alternative pathways (CH100 and AP100).

Protein expression in leucocytes may form an important part of the diagnostic pathway in certain conditions. Flow cytometric analysis of WAS protein expression in leucocytes aids the diagnosis of WAS [33] and, in the case of WAS and XLT, there is correlation between the amount of protein expression and clinical prognosis. Degree of abnormal protein expression does not always correlate with prognosis, however, and this is particular true in XLP.

It is now frequently possible to confirm a genetic diagnosis which may not alter management but may enable a more accurate prognosis to be given and also further information about recurrence to be given to parents and wider family.

The theme throughout this review has been the infective clues and these should never be understated; they may well be the first indication of an underlying problem and provide significant information as to what that problem is.

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Mycetoma Caused by *Madurella mycetomatis:* A Completely Neglected Medico-social Dilemma

15

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Abstract

Mycetoma is a debilitating disease with a highly particular geographical distribution. The mycetoma belt circles the entire world just above the equator and defines the region with the highest prevalence and incidence. Although the disease is seen in Central America, India and all across Africa, Sudan seems to be the homeland of mycetoma. Mycetoma is an infectious disease caused either by bacteria (actinomycetoma) or true fungi (eumycetoma).

In Sudan most cases are caused by the fungal species *Madurella mycetomatis*. The precise natural habitat of this fungus is still an enigma, but its DNA can easily be found in soil and plant samples in endemic areas. Although the entire human population in these areas are in regular contact with the fungus, most individuals are unaffected. Thus mycetoma is an ideal clinical and experimental model system for the study of host-pathogen interactions. Also, given its relative importance locally, improvements in clinical and laboratory diagnostics and knowledge of the epidemiology of the disease are badly needed. This chapter describes the current state of affairs in the field of eumycetoma caused by *M. mycetomatis*. The value of laboratory research on this disease and future perspective for control and prevention of the infection are discussed.

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15.1 Introduction

Mycetoma, regionally also known as "Madura foot", is a chronic granulomatous inflammatory disease of the extremities in particular [1, 2]. However, no single bodily part is fully exempt. First described in a patient from India [3], the infection can be caused either by bacteria (actinomycetoma) or fungi (eumycetoma) [4]. It is characterized by the presence of a chronic sub-
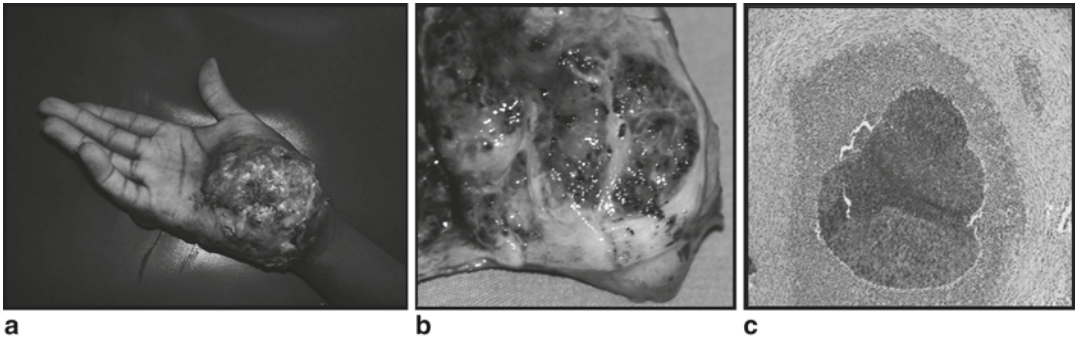
Fig. 15.1 Mycetoma from lesion to microscopic pathology. **a** Picture of the hand of a patient suffering from myce-toma infection with *Madurella mycetomatis*. Note the *yellowish*, purulent exudate that is characteristic for this disease. Magnification will show the presence of the *black* grains as well. **b** Surgically removed mycetoma lesion in which the macroscopic *black* grains are more clearly visible. **c** Histological analysis of a tissue-embedded *black* grain. The central cement like structure can be seen to be completely surrounded by infiltrated neutrophils

Fig. 15.2 Cranial mycetoma in a young child. **a** Exterior part of the lesion. **b** MRI picture showing the internal protrusion of the lesion
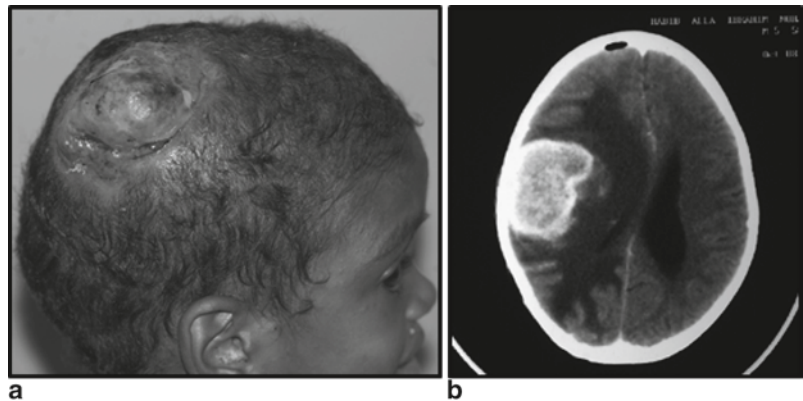
cutaneous mass in which sinuses will develop (Figs. 15.1 and 15.2). Through these sinuses, purulent material and grains of fungal or bacte-rial origin are emitted. Grains can have varying colors, depending on the causative agent. Over-all, as a preliminary diagnostic indicator, bacte-rial grains are white, yellow or red while fungal grains are mostly black or white. Although the presence of grains is characteristic of mycetoma, there is still limited understanding of the physi-cal nature and physiological or pathogenic role of these grains [1, 5].

Most of the causative agents are believed to be soil-dwelling micro-organisms which are acci-dently introduced into the host, possibly follow-ing minor trauma such as a thorn prick [6]. Once inside the body, the causative agent starts to pro-liferate very slowly. The first symptoms may take several months, even years to become palpable. The mycetoma infection itself is painless, which is why most patients don't seek early medical help, doing so only when the lesion becomes large and the bones become affected. Due to open sinuses and frequent poor hygiene of the open wound, most patients are primarily troubled by other bacterial super-infections, which do cause additional discomfort [7].

Overall, mycetoma is a devastating disease causing obvious physical scars as well as socio-economic and psychological ones. Several fea-tures of mycetoma are presented to help highlight this significantly neglected and very serious trop-ical disease.

15.2   The Population at Risk and Approaches to Management

Males are more susceptible than females and children may be affected as well. Late in the disease, dissemination to other bodily locations and severe local tissue destruction (including that of bone) is frequently observed [5, 8–10]. The endemic areas are diverse but mainly tropical. People in large parts of Central Africa, Central America and India are subject to increased risk of infection [1]. Therapy for mycetoma is usually based on local experience and relatively unsophisticated practice as no therapeutic consensus has yet been reached [8, 11, 12]. Treatment is largely guided by relatively subjective clinical observations as laboratory-based diagnosis to guide therapy is lacking. Bacterial actinomycetoma can be treated with antimicrobial agents alone with a high success rate. However, fungal mycetoma is not effectively treated with antifungal agents alone. Surgical intervention (debridement, wide local excision or amputation) is required in combination with post-surgical chemotherapeutic treatment. Success rates are higher when treatment is started early in the infection. Long courses of high doses of antifungals are needed (ketoconazole or itraconazole at 400–800 mg per day) for durations which vary from case to case [13–15]. Treatment success can only be defined on the basis of regular long term follow up to check for recurrences which are rather frequent. This long and unpredictable course makes therapeutic trials difficult to perform. Despite the fact that large numbers of people living in endemic areas risk being infected on a daily basis, essentially nothing is known on disease prevention. More detailed knowledge of the pathophysiology of this disease is badly needed.

15.3   Causative Agents

Mycetoma can be caused by a large variety of micro-organisms, both bacterial and fungal in nature. The causative bacterial species mostly belong to the genera *Actinomadura* spp, *Acti-nomyces* spp, *Nocardia* spp and *Streptomyces* spp [1, 4]. There is even more taxonomic diversity among the fungal species that are capable of causing mycetoma. These include species of *Acremonium, Aspergillus, Curvularia, Fusarium, Leptosphaeria, Pyrenochaeta* and several others [11, 16–18]. New and unexpected causative agents are still being discovered on a regular basis. One of the most prominent genera is that of *Madurella* spp, represented in particular by its best known species, *Madurella mycetomatis.* This species is most prevalent in several areas where mycetoma is a clearly endemic disease. For instance, in Central Sudan most mycetoma cases are due to *M. mycetomatis* [19, 20]. This chapter focuses on *M. mycetomatis* mycetoma as it is the best studied epidemiological, clinical, patho-physiological and host response model system to date.

15.4   Mycetoma in Children

Mycetoma including those cases caused by *M. mycetomatis* is thought to be relatively rare in children although precise prevalence data from endemic regions are lacking and should be undertaken. Fortunately, recently a large case series has been presented [21] allowing several important conclusions to be drawn but also raising additional questions.

As in adults, boys stand a larger chance of acquiring mycetoma than girls. Of the patients treated, approximately 73 % were boys and in the Central Sudan region where this study originates 80 % of all patients were infected with *M. mycetomatis.* Interestingly, 15 % of the junior patients (age 4–17 years, average 13.7 years) had a family history of mycetoma. Foot, knee, hand, head and neck were the most frequently affected areas and the lesions showed the characteristic sinuses, local sweating, lymphadenopathy and varicose veins. Bone involvement was as frequently observed as in adults and after apparently successful chemical and surgical treatment 18 % of patients still suffered from a relapse. Morbidity was high and in 2 % of the

patients a (partial) amputation of a limb had to be performed.

There are several practical problems relating to management of mycetoma in children. Presentation is characteristically late, not only because of fear of the often mutilating surgical treatment but also because of children's complete dependence on adult support and frequent financial constraints. Approximately 45 % of the children had undergone some sort of prior treatment by local physicians or less qualified (para-)medical personnel including people specialized in traditional medicine. In all cases, such interventions had been unsuccessful and in many cases had apparently worsened rather than improved the disease or its prognosis. After treatment, both clinical follow-up and compliance with chemotherapy were problematic and certainly not systematically achieved. Frequent travelling to and from the clinic is a major obstacle for many patients, many of them living in remote areas. For the same reason, chemotherapy was often irregular and incomplete. All these problems are aggravated by the lack of financial resources and medical insurance. In addition, the children suffered from obvious and serious secondary socio-economic consequences: with frequent abandonment of schooling and psychological issues including depression, despair and anxiety.

Other factors may contribute to the clinical impact of mycetoma. Two very important issues are hygiene and protection from infection, which are associated with the nearly complete lack of health education among many affected individuals. There is limited knowledge about disease prevention, recognition and optimal treatment. There is clearly scope for improved education and the necessary tools are not especially costly and may already be available. Health education campaigns can also reach remote areas although this can present challenges. In addition, more advanced research into the pathophysiology of the disease is urgently required as discussed in the remainder of this chapter.

15.5 Diagnostic Features of *Madurella mycetomatis*

15.5.1 Direct Identification in Tissue Samples

For diagnosis of *M. mycetomatis* mycetoma fine needle aspirates can be taken directly from the lesion. Based on histological analysis of surgical biopsies, within the lesion there are slowly maturing grains in which, for instance, Grocott staining can help visualize fungal material [8, 22]. The mycelium is melanised and the developing grain is usually surrounded by a layer of neutrophils. However, in most laboratories fungal culture of the grains that are expelled from the lesion through the sinuses is more common practice than histopathology which is more difficult. The fungal grains are easy to obtain and can be conveniently inoculated on yeast- and fungus-specific artificial growth media. However, cultures may need to be incubated for weeks in order to be confidently designated as negative. Furthermore, mis-identifications do occur on a regular basis. Reliable serological tests do not exist at the moment although some immunodiffusion tests have been described as has a newly developed but not yet clinically employed ELISA system targeting the human antibody response to the translationally-controlled tumor protein (TCTP) of *M. mycetomatis* [23–27]. This latter test employs recombinant TCTP or *in vitro* synthesized TCTP-specific peptides, derived from the first *M. mycetomatis*-specific antigen so far identified. Further novel *M. mycetomatis*-specific antigens are currently being identified and serological testing may be significantly improved in the near future [28].

15.5.2 Growth-based Testing

M. mycetomatis is a fungus that grows slowly on artificial growth media. It produces dark, brown-to-black colonies consisting of dense, melanised and, interestingly, always sterile mycelium. Phase variation with respect to melanisation is

frequently seen: in these cases only a sector of the colony is melanised with the remaining taking on a brown to beige color. Colonies may acquire spectacular three dimensional structures and the color may vary regionally within a single colony. There is extensive phenotype variation and it is impossible to speciate *M. mycetomatis* reliably on the basis of morphological features of the colony. In contrast to the variability in the colony, microscopic morphology is rather uniform. No distinguishing sporulating structures are observed. *M. mycetomatis* is a sterile fungus, which make determination based on microscopy almost impossible [1, 29]. It appears that many fungi were wrongly identified as *M. mycetomatis* in the past [18] and culture based diagnostics needs to be complemented with alternative information including for instance molecular data.

15.5.3 Molecular Testing

Since microbiological, growth-based diagnostics is not unequivocal, new technologies have been explored for their usefulness. One example is molecular testing by PCR. Amplification of the ribosomal internally transcribed spacer (ITS) regions has led to the development of a specific PCR restriction fragment length polymorphism (RFLP) test [17, 19]. This test proved to be important in speciation of the cultured fungus, but also turned out to be very useful in the detection of (non-cultivable) *M. mycetomatis* antigens in soil samples [6]. The test revealed that as a species, *M. mycetomatis* behaves homogeneously in the diagnostic PCR tests and that in case of soil samples from endemic regions as many as 25 % of samples can be scored positive for *M. mycetomatis* DNA at least [6]. The same was documented for acacia thorns indicating the *M. mycetomatis* is environmentally widely-spread. Soil samples from non-endemic areas were negative in these tests.

Another form of molecular testing that can be applied to DNA extracted from *M. mycetomatis* biomass is amplified fragment length polymorphism (AFLP) analysis [30]. This is a technique that helps to PCR-amplify DNA restriction frag-

ment subsets selectively [31]. Variability in the length of such fragments due to sequence variation in the restriction enzyme recognition sites (for example between two isolates of the same species) will define whether or not a species is clonal or genetically diverse. In turn, this may help to define patterns of epidemiological spread of microbes in general and in our case *M. mycetomatis* in particular. Although this technique has not been applied very often in fungal mycetoma research, one study showed that *M. mycetomatis* is genetically quite stable but that on the basis of minor genetic polymorphisms it could be concluded that regional epidemic spread of certain genotypes of *M. mycetomatis* was occurring [30]. Whether such regional clones differ in disease causing potential is currently not known.

15.5.4 Antifungal Susceptibility Testing and Response to Treatment

In vitro testing for anti-fungal resistance can help tailor adequate chemotherapy for patients. Using newly developed tests it has been demonstrated that *M. mycetomatis* remains highly susceptible to the azole-group of antifungal agents [15, 32]. Low MICs were obtained for ketoconazole, itraconazole, voriconazole and posaconazole. Also low MICs were obtained for terbinafin [32, 33]. Moderate susceptibility was documented for amphotericin B and fluconazole whereas all isolates were completely resistant to 5-flucytosine and the echinocandins studied (caspofungin, anidulafungin and mycafungin) [34]. MICs became elevated for the azoles ketoconazole and itraconazole when melanin was added to the culture medium, suggesting that melanin may have a biological function in shielding the fungi from chemical attack. Interestingly this was not noted for voriconazole. However, although the fungus may appear fully susceptible *in vitro*, this may not predict the *in vivo* infectious setting, especially since the fungus organizes itself into grains *in vivo,* while *in vitro* only hyphal fragments can be used in susceptibility testing. Indeed, clinical trials with

ketoconazole, itraconazole, posaconazole and terbinafin, show that variable and even conflicting results are obtained with these antifungals. As an example in a recently published clinical trial in 13 *M. mycetomatis* infected patients treated with 400 mg/day itraconazole, a complete response was observed in only one patient. A partial response was noted in nine and in three the disease remained unchanged. One patient had a recurrence after 18 months [13]. Similar results were obtained with ketoconazole [35], posaconazole [36] and terbinafin [37]. Studies with additional compounds such as artemisinin and tee trea oil have been performed and several of these studies identify new therapeutic opportunities [37].

15.6 *Madurella mycetomatis* Virulence Factors

Virulence can be defined as the ability of an infectious agent to cause disease in an affected human individual [38]. This usually is a multistep process, which ranges from attachment of microbial cells to colonization of the host and, later on, to host invasion and tissue damage. Very little is known on the mechanisms leading to human infection by *M. mycetomatis.* However, some information is available on a limited number of molecules that could play a role in mycetoma pathogenesis.

15.6.1 Melanin

Melanin has been shown to be a very important content component of the mycetoma grain. Furthermore as early in 1978 it was shown that as the secreted pigment of mycetoma, melanin was able to transform collagen into a hard matrix, resembling the material of which the fungal grain is composed [39]. Melanin is a complex series of different molecules which can be synthesized via different pathways. The biochemistry is complex but essentially there are three pathways known that lead to the synthesis of the different forms of melanin: pyo-, dopa- and DHN-melanin [40].

Using pathway inhibitors, it has been shown that *M. mycetomatis* produces primarily pyo- and DHN-melanin [41]. Melanin is known to protect these micro-organisms against UV-radiation, enzymatic lysis of the fungal cells, oxidant activities and killing by alveolar macrophages [42]. In *M. mycetomatis* complementary *in vitro* testing demonstrated that melanin has protective effects against the activity of antifungal agents as well. For instance, melanin binds effectively to amphotericin B, thereby potentially blocking its antifungal activity. However, there is a need for further studies into the influence of melanin on the susceptibility to antifungals and, hence, therapeutic success.

15.6.2 TCTP

As indicated above, TCTP was the first *M. mycetomatis* molecule to which a specific human response was defined [23]. TCTP is found in many eukaryotes and it has been shown to bind the anti-malaria drug artemisinin. It is thought to be involved in cell cycle control, various stress responses, histamine release and interleukin responses. Furthermore a TCTP variant of the fungus *Cladosporium herbarum*, can induce release of histamine from human basophils, indicating that TCTP may have immunomodulating effects [43]. Although the precise function of TCTP in *M. mycetomatis* has not yet been defined, several findings suggest it may play a role in mycetoma development. For one, the protein is only expressed in the very young grain and not in aged grains, unlike the other two antigens known for *M. mycetomatis*: fructose bisphosphate aldolase (FBA) and pyruvate kinase (PK) [28]. Such stage-specific expression may be associated with invasiveness. Serum anti-TCTP antibody testing also shows some interesting features. It was shown that there were significant differences between the serum response in patients versus controls, as was also noted for FBA. This suggests that immune responses are only or are primarily induced after infection. Additional research into the pathogenic relevance of this molecule is certainly warranted.

15.7 Host Susceptibility to *Madurella mycetomatis* Infection

Why do some people develop mycetoma and others not? PCR testing has revealed that in endemic areas *M. mycetomatis* is quite ubiquitous in soil [6]. Furthermore, most indigenous people appear to have antibodies against *M. mycetomatis* [44]. This suggests that regional inhabitants may all be in regular contact with the fungus. Still only a fraction of individuals will become infected in the end. Also the observed differences between frequency of infection in males and females (despite presumably similar exposure) [1], suggests that there must be differences in host susceptibility to infection. There are some conflicting reports about the role of the immune status of the susceptible population. As early as in 1977, local researchers showed that there was a partial impairment of the cell-mediated immune (CMI) response in patients with severe mycetoma [45]. This impaired CMI hypothesis was supported by the fact that mycetoma could more easily and successfully be induced in athymic mice than in immune-competent mice [46]. Furthermore mycetoma was more rapidly induced in immune-suppressed goats than in healthy goats [47]. However, both Bendl *et al.*, and Ahmed *et al.* did not confirm this finding: they showed that mycetoma could be induced in immune-competent animals [48, 49]. In the Mycetoma Research Center in Khartoum, Sudan, it was noted that mycetoma is generally seen in immune-competent individuals with no apparent immunodeficiency. Nevertheless, it was noted, that HIV-infected individuals show a tendency towards accelerated mycetoma progression [50].

Although there were no obvious immune defects noted in mycetoma patients, that does not mean that there are no subtle immunological differences between the people who will develop mycetoma infection and who will not. Most of these differences will have a genetic basis and a number of recent studies have reported several genetic markers associated with the development of fungal mycetoma [44, 51].

15.7.1 Neutrophil Function

Mycetoma is a clear example of an inflammatory disease. The combination of histochemistry and histopathology has already shown the obvious infiltration of a large number of neutrophils around developing and fully-developed black *M. mycetomatis* grains [1, 22]. This suggests that neutrophil function may be an important feature of host resistance or susceptibility to mycetoma. However, neutrophil function is complex and the anti-infectious response consists of a large number of activating and inhibitory steps implying that many different genes may contribute to such differences in susceptibility [52]. Genetic comparison of eight genes in these various maturation cascades has helped to identify different marker gene polymorphisms [44]. Among these were complement receptor 1 (CR1), the chemokine interleukin 8 (CXCL8), the interleukin 2 receptor, the chemokine receptor CXCR2, nitric oxide synthase NOS2 and TSP4. Although genetic studies may be biased, for instance based on the selection of patients or the inclusion of inadequate control individuals, functional testing can provide additional support. For instance for interleukin 8 (CXCL8), the genotypes documented in association with mycetoma were correlated with higher CXCL8 production which was also demonstrated phenotypically in the sera of these patients. For the nitric oxide synthase, it was shown immunohistochemically that the enzyme was actively produced at the site of infection. In addition, it was shown that the serum nitrite/nitrate levels were significantly lower in the serum of patients than in the serum of controls. Taken together these data support the concept that variations in neutrophil function are associated with susceptibility to mycetoma.

15.7.2 Cathechol-O-methyltranferase and Cytochrome p450 Subfamily 19 Genes

Since the higher incidence eumycetoma caused by *M. mycetomatis* in males cannot easily be

explained by behavioral differences between the local men and women, other factors, including sex hormones, could be associated with susceptibility. To monitor for possible deficiencies in hormone synthesis among mycetoma patients, we investigated the types and allele frequencies of the genes encoding for catechol-O-methyl-transferase (COMT), cytochrome p450 subfamily 1 (CYP1B1), cytochrome p450 subfamily 17 (CYP17), cytochrome p450 subfamily 19 (CYP19) and hydroxysteroid dehydrogenase 3B (HSD3B) [51]. Significant differences in allele distribution were demonstrated for CYP19 ($p=0.004$) and COMT ($p=0.005$), as well as gender dimorphism for both CYP19 and COMT polymorphisms. The COMT polymorphism was associated with mycetoma lesion size as well. The genotypes obtained for COMT and CYP19 were connected with higher 17β-estradiol production, which was confirmed by significantly elevated serum levels of 17β-estradiol in male patients. In contrast, lower levels of dehydroepiandrosteron (DHEA) were found in mycetoma patients. The *in vitro* growth of *M. mycetomatis* was not influenced by 17β-estradiol, progesterone, DHEA and testosterone excluding the possibility that the catabolism of host hormones plays a role in susceptibility to mycetoma. Apparently, the differences in hormone levels we noted between mycetoma patients and healthy controls were unlikely directly to affect the fungus itself. Indirect effects on the patients' hormone regulated immune states hypothetically form the more likely explanations for their association with mycetoma susceptibility.

Given the above observations and the relative ease with which genetic markers for mycetoma predisposition were obtained, a good strategy for future work may be Genome Wide Association studies [53, 54]. These would entail the comparison of millions of genome polymorphisms that may be consistent between patients and control generating a catalogue of factors that are potentially relevant to mycetoma development and severity. This approach may be helpful in developing innovative prophylactic, diagnostic and therapeutic regimens.

15.8 Concluding Remarks and Future Prospects

Our insight in microbial biology and infectious diseases has been enormously boosted by recent technological advances in laboratory methodologies. The first and foremost of these are the next generation sequencing (NGS) technologies [55, 56]. In all fields of infectious disease research, the potential impact of such technologies is becoming obvious. Bacterial whole genome sequences are numerous and for all medically relevant microbes several strain-specific genome sequences are publicly available [57]. This has led to the identification of core versus variable genomes and the characterization of new virulence genes and mechanisms. For *M. mycetomatis* such sequences are not yet available but may be defined soon. This may enhance our insight into fungal biology and help develop novel tools and strategies for prevention and treatment of disease. These fungal genome data need to be complemented with additional host genomic markers that correlate with disease susceptibility or severity. The same genome sequences used for bacterial whole genome assembly will be useful in this field as well. In the end a combination of fungal and host genome polymorphisms may allow for the detailed study of genome-genome interaction in mycetoma, which will further refine our strategies for prevention and cure.

However, it has to be noted that mycetoma is a disease of the poor and although it is a severe disease its overall impact does not compete with those, for example, of HIV or malaria. The number of mycetoma researchers worldwide is extremely small. It is certain that the field will progress significantly over the years to come but the main question still is whether the additional knowledge will attract the funds needed to facilitate real practical and clinical interventions. Until then, we may need instead to consider the implementation of simpler methods including health education to increase medical awareness among the populations at the highest risk of acquiring infection. The provision of footwear in endemic

areas may be another simple intervention worth contemplating.

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Elucidation and Clinical Role of Emerging Viral Respiratory Tract Infections in Children

Inge Ahout, Gerben Ferwerda and Ronald de Groot

Abstract

Acute respiratory viral infections (ARVI's) are the most common infectious disease in humans. With the appearance of molecular techniques the recovery of viruses has dramatically increased. Nowadays virologists can quickly discriminate virological families and related viruses from emerging viruses and consequently identify novel viruses. Many new respiratory viruses have been identified in children in the past 15 years. In this review we shortly discuss novel respiratory viruses and their pathogenic role in pediatric respiratory disease. Advantages and drawbacks of the technique and our current knowledge will be discussed. We will conclude this review with a general discussion on the future role of molecular diagnostic virology in the clinic.

16.1 Introduction

16.1.1 General (Respiratory Viruses in Pediatrics)

Acute respiratory viral infections (ARVI's) are the most common infectious disease in humans. They occur more frequently in children than in adults (6.1 episodes per year under the age of 1, 3–6 episodes per year between the age of 1–5 and 2.4 episodes per year between the age of 15–19). Disease severity depends on age, underlying condition and type of virus. ARVIs account for huge numbers of doctor's visits and days lost from work and school. They are a leading cause of global mortality and morbidity in children. Moreover, respiratory viral infections are an important driver of unnecessary usage of antibiotics. Unfortunately prevention and treatment of the majority of respiratory virus infections is not possible with the exception of influenza [1].

Although much research has been done on the epidemiology and burden of viral respiratory tract infections the size of the problem is underestimated. Due to the lack of routine testing for (multiple) viruses and the limitation that a majority of infected patients will not visit a doctor.

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16.1.2 General Introduction Molecular Techniques (Discovery Novel Viruses)

With the appearance of molecular techniques the recovery of viruses has dramatically increased. Before their use approximately 50–80 % of the viral tests remained negative [2]. Due to the introduction of PCR and the discovery of novel viruses this proportion decreased to 3–15 %. However, such recovery rates are largely dependent on the selection of the patient group [3–5]. The combination of high sensitivity, multiplex options and quantification was essential for some of the new insights in viral epidemiology. This could not have been achieved with conventional viral diagnostics such as culture and immunefluorescence assays (FDA).

The identification of respiratory viruses in a clinical context can also guide diagnostic and treatment strategies. Bonner et al. [6] revealed that a known viral aetiology of disease results in decreased use of additional tests such as X-rays or blood examination, shorter hospital admission and less frequent use of antibiotics. However, molecular diagnostics have also created new dilemmas. For example, the identification of respiratory viruses in asymptomatic children, the occurrence of many viral co-infections, concerns about the pathogenic capacity of certain viruses and the value of quantitative measurements.

16.1.3 General Introduction on Emerging Viral Diseases

Some of the advantages of the new genetic (e.g. sequencing) and molecular techniques became clear during outbreaks of novel emerging viruses. Emerging viruses can be classified as (1) previously unknown viruses or (2) previously known viruses that have significantly increased in incidence [7]. Nowadays virologists can quickly discriminate virological families and related viruses from emerging viruses and consequently identify novel viruses.

The introduction of molecular diagnostics in medical virology has led to the identification of

Table 16.1 Emerging viruses from the last 2 decades

Virus	Family	Year of discovery
Hendra-/NipahV	Paramyxovirus	1995
AIV's	Influenza virus	1997
hMPV	Paramyxovirus	2001
SARS-CoV	Coronavirus	2003
HCoV-NL63	Coronavirus	2004
HCoV-HKU1	Coronavirus	2005
HBoV	Parvovirus	2005
HPeV4	Parechovirus	2006
HPeV5	Parechovirus	2006
HPeV6	Parechovirus	2007
KIV/WUV	Polyomavirus	2007
H1N1V	Influenza virus	2009

many new respiratory viruses in children in the past 15 years (Table 16.1). However, the pathogenicity of these viruses is not always clear and the clinical relevance is often poorly understood. Fredricks and Relman proposed seven rules which are necessary to demonstrate the causative relationship between a virus and disease. These rules are based on Koch's postulates and were adapted for nucleic acid based detection methods, location of the pathogen and quantification (Table 16.2) [8, 9]. These rules can help to interpret research on the role of novel respiratory viruses in disease and guide future research. It should also be stated that the clinical relevance is in some cases apparent, without extensive research to fulfil all requirements.

In this review we briefly discuss novel respiratory viruses and their pathogenic role in pediatric respiratory disease. We will conclude this review with general discussion on the future role of molecular diagnostic virology in the clinic.

16.2 Henipavirus (1994–1998)

16.2.1 Hendra Virus

The Hendra virus was first detected in a disease outbreak in 1994. It initially presented with a new respiratory disease in horses that was transmitted to two persons one of them died [10]. The virus belongs to the genus of *Henipavirus* within

Table 16.2 Koch's postulates adapted for viral infections [8, 9]

A nucleic acid sequence belonging to a putative pathogen should be present in most cases of an infectious disease. Microbial nucleic acids should be found preferentially in those organs or gross anatomic sites known to be diseased, and not in those organs that lack pathology
Fewer, or no, copy numbers of pathogen-associated nucleic acid sequences should occur in hosts or tissues without disease
With resolution of disease, the copy number of pathogen-associated nucleic acid sequences should decrease or become undetectable. With clinical relapse, the opposite should occur
When sequence detection predates disease, or sequence copy number correlates with severity of disease or pathology, the sequence-disease association is more likely to be a causal relationship
The nature of the microorganism inferred from the available sequence should be consistent with the known biological characteristics of that group of organisms
Tissue-sequence correlates should be sought at the cellular level: efforts should be made to demonstrate specific in situ hybridization of microbial sequence to areas of tissue pathology and to visible microorganisms or to areas where microorganisms are presumed to be located
These sequence-based forms of evidence for microbial causation should be reproducible

the family of the *Paramyxoviridae* family. It was initially named morbillivirus and later re-named Hendra virus (HeV) after the suburb where the outbreak occurred [11, 12]. The virus itself is not very contagious. It spreads through direct contact between horses or during intensive contact between humans and severely ill horses. The animal reservoir appears to be the Flying-fox, in this population the Hendra infection is largely subclinical. The breading season of Flying-foxes is a risk period for spread and the human risk group is defined as people with close and intensive contact with horses. Up till now fourteen outbreaks have been reported [13]. In five of these outbreaks humans were involved, resulting in five deaths. So far, only two persons survived an infection. The case fatality rate (CFR) is over 50 % [13]. HeV in humans causes, after an incubation period of 5–21 days, a severe influenza like disease (fever, myalgia and headache) which can progress to

pneumonia, respiratory failure and death [10, 14]. An infection can also result in encephalitis with headache, fever and drowsiness. The encephalitis can occur after initial recovery from the illness. The Hendra virus genome is readily detected in several materials, e.g. blood, urine, nasal- and oropharyngeal swabs by RT-PCR. Next to this standard detection method the virus can be cultured in several cell lines, where it forms syncytia upon infection. ELISA serological tests are used for screening, however their diagnostic sensitivity is not yet established. Immunofluorescense assays and serum neutralization methods can also be used [13, 15].

16.2.2   Nipah Virus

In 1998 and 1999 two large outbreaks of respiratory disease in pigs and humans occurred. In Malaysia and Singapore 106 people died [16]. The causative agent had large similarities to the HeV and is the second member of the genus of *Henipavirus* within the *Paramyxoviridae* family. It was named Nipah virus (NiV) after the location of the first human case [17]. This virus had already caused respiratory disease in pigs until late 1996. Like HeV it shares the bat as a natural reservoir. NiV virus is very contagious among pigs and spreads through the respiratory route or directly by the transport of infected pigs. Initially humans became infected via direct contact with pigs, although food borne transmissions were also reported. Initially the case fatality rate was 38.5 % [14, 18]. Since the initial outbreak almost yearly new outbreaks emerged in Bangladesh and India causing fatal encephalitis in humans. Notably, the CFR's of these outbreaks increased to 92 %. Also the transmission changed: starting from pigs, soon cows could transmit the virus. Later human to human and nosocomial transmission was demonstrated [19–21]. It has been hypothesized that there were multiple introductions of viruses in the human population, explaining the unique genetic signature of isolated viruses nowadays [18]. These genetic differences may be the reason for the increase in CFR and differences in clinical manifestations

and transmission. The clinical manifestation of a Nipah infection differed per outbreak. Incubation periods differ from an average of 2 weeks in Malaysia to 1 week in Bangladesh. The infection can be asymptomatic, but often starts with influenza-like symptoms of fever, headache, myalgia, vomiting and sore throat. Patients can recover or develop signs of encephalitis or sometimes atypical pneumonia or acute respiratory distress. In severe cases the encephalitis includes the brain stem or progresses to a coma within 24–48 h [18, 19, 22]. Around 20 % of the cases are left with residual neurological symptoms, including personality changes. In comparison with outbreaks in Malaysia and Singapore the Bangladesh and Indian patients experienced more profound respiratory symptoms with case rates of 14, 27, 70 and 51 %, respectively [19]. A Nipah infection can be diagnosed in serum urine and cerebrospinal fluid (CSF) by RT-PCR [23]. Also culture in cell-lines, ELISA for anti-HeV IgG and IgM in serum and CSF, serum neutralization assays or immunofluorescence assays are used [18, 19, 24, 25].

Patients with both Hendra and Nipah virus infections are treated supportive, antiviral therapy is not effective [22]. Prevention is based on careful hygiene, quarantine and safe disposal of animal carcasses [13, 14]. Currently, there are no vaccines available. However, several therapeutic agents seem effective *in vitro* and in some animal models [18].

16.2.3 Avian Influenza Virus (1997)

The first cases of avian influenza virus (AIV) infection were reported in 1997 in Honk-Kong [26]. This influenza A (H5H1) originated completely from strains circulating in wild birds and poultry [27]. The avian influenza virus undergoes rapid genetic and antigenic evolution reflected by the occurrence of different clades with distinct phenotypes [28, 29]. The majority of human cases had direct contact with poultry or could be related to outbreaks in wild birds [20]. There is limited transmission from human to human, although some epidemiological studies suggest it is possible [30]. The median age of patients is around 18 years and the mortality rate is extremely high between the age of 10 and 19 years (61 %). Yearly H5N1 outbreaks in humans have been reported in Asia, Africa and Eurasia [31]. These epidemics are all related to outbreaks of avian influenza in wild birds or poultry during the colder seasons [32, 33]. The incubation period of H5N1 is estimated to be 2–7 days [34]. The disease typically manifests as a severe pneumonia which often progresses to respiratory failure and death within 10 days (case fatality rate up to 90 % in children). It appears that in children cases may occur without pneumonia. Detection of viral RNA by (RT-) PCR is the best method for the diagnosis of H5N1, preferably using throat swabs [35]. Because of genetic variably of the virus, primers need to up-dated frequently. The available immune-assays for detection of H5N1 are not sensitive enough for clinical purposes and cannot differentiate between human and avian subtypes of influenza A. Seroconversion after 2–3 weeks can be used to confirm H5N1 infection and can be used for epidemiological studies [35]. Early treatment with oseltamivir is recommended based on some evidence that it increase survival rates [36]. There are differences in susceptibility to oseltamivir between the different clades of H5N1 circulating in different parts of the world. Combination of oseltamivir with amantidine can be given if the circulating H5N1 is susceptible to both agents. Currently, it is possible to produce vaccines that inactivate H5 influenza A strains. However due to the circulation of different clades and the rapidly changing antigenicity of H5N1 the need for the development of a new vaccine remains [34, 37].

16.2.4 Metapneumovirus (2001)

The human metapneumovirus (hMPV) was first discovered in the Netherlands in 2001 from a databank of samples from children with respiratory tract infections [38]. hMPV belongs to the genus *metapneumovirus* within the family of *Paramyxoviridae*. It is related to respiratory

Table 16.3 Symptoms and diagnosis of hMPV mono infections in literature

Symptoms/diagnosis	Spread in literature[a]
Fever[b]	36–80
Cough	67–99
Rhinitis	72–90
Wheezing	10–73
Respiratory failure	8
Oxygen 90 %	32–85
Pharyngitis	24–66
Bronchitis	1–68
Bronchiolitis	11–51
Pneumonia	3–65
Otitis media	16

[a] Heikkinen et al. [121]; Aberle et al. [122]; Mullins et al. [123]; Chen et al. [124]; Manoha et al. [125]; Williams et al. [126]; Bosis et al. [127]
[b] Different definitions varying from >37.5 to >39 °C

syncytial virus, both belonging to the *pneumoviridae* sub-family. In both retrospective and prospective studies it has been shown that hMPV can be detected in 3.9–14.8 % of respiratory samples from children with respiratory disease [39]. This wide range reflects differences in the tested populations and the level of care. Co-infections with other viruses occur in 15–30 % [40–42]. hMPV is detected in up to 4 % of nasopharyngeal aspirates from healthy children, although percentages of less than 1 % are also frequently published [43]. Serological studies showed that all children by the age of 5 years had been in contact with the virus and that it has been circulating in the human population for over 50 years [38]. It has a seasonal occurrence with a peak incidence just after the influenza and RSV season [43]. Spread is thought to be via direct or close contact with respiratory secretions from an infected person with an incubation period of 3–5 days. Re-infections occur frequently in children, although symptoms are less severe [44]. Symptoms associated with hMPV infections are comparable with RSV (see Table 16.3). hMPV infections are, after RSV, the most frequent cause of bronchiolitis in young children and account for 5–15 % of all hospital admissions [45]. Hospitalization rates are highest among 6–12

month old children, remarkably older than for RSV [46]. There is an association between severe hMPV infection (bronchiolitis) and the development of wheezing in childhood [47].

hMPV can only be cultured in specific cell lines under specific conditions and is time consuming; therefore it has no role in a clinical setting. Real time PCR is the most sensitive test for hMPV detection in NPA and swabs [48] and is therefore the common method in clinical and research settings. RT-PCR also provides semi-quantitative information of the viral load (Ct value), which can be used to monitor treatment in a research setting [49]. Immunofluorescence assays are available for rapid detection of the virus in respiratory specimens; however, these tests are less sensitive than RT-PCR. Serology for hMPV has little additive value in the clinic because most children are seropositive in early childhood. Currently no vaccines against hMPV are available, though several candidates are being pursued [45]. Ribavirin, antiviral therapy, is effective *in vitro* against hMPV, though clinical data are sparce. Currently new therapies such as fusion inhibitors and siRNA's are being tested in murine models [45].

16.2.5 Coronavirus (2003–2005)

Human corona viruses related to respiratory disease, 229E and OC43, have been known since the 1930s. They were recognized as the second most common cause of the common cold in humans [50–52]. A new strain of human coronavirus was identified in 2004 from a respiratory sample of a 7 month old infant with bronchiolitis and named NL63 (HCoV-NL63) [53]. The HCoV-NL63 belongs to the genus Coronavirus within the family of Coronavidae. In retrospective cohort studies HCoV-NL63 have been identified in 1.7–9.3 % of respiratory samples from children with respiratory symptoms and occurs worldwide [39]. The virus is often found in combination with other respiratory viruses (57 %) [54, 55]. Peak incidence is found in the winter months and the incubation period is estimated

2–5 days [56, 57]. HCoV-NL63 is associated with mild upper respiratory tract symptoms and less frequent with severe lower respiratory tract symptoms such as bronchiolitis [58]. Some studies have reported an association with croup [55, 59]. HCoV-NL can be detected in respiratory specimens by RT-PCR which is the first choice for diagnosis. Immunoassays are available for rapid detection and distinction of different HCoV strains [60]. Different cell-lines are permissive for viral culture and used in a research setting. Currently no anti-viral treatment against HCoViNL63 is available, although several inhibiting compounds have been identified [61].

A second novel human coronavirus was identified in 2005 in a 71-year-old man with pneumonia in China and named HKU1 after the Hong Kong University where it was found [62]. In a retrospective cohort studies the HCoV-HKU1 was identified in 1–3.1 % of respiratory samples in which no other virus was detected, from children with upper and lower respiratory symptoms [39] with a higher incidence in children younger than 6 months. The peak incidence of HCoV-HKU1 is in spring, early summer and winter with an incubation period of 2 days [63]. HCoV-HKU1 is mainly associated with upper respiratory tract symptoms in children and occasionally with pneumonia and bronchiolitis [64]. The first choice of assay for detection in respiratory specimens is RT-PCR. Coronaviruses exhibit substantial genetic variability hampering the development of pancorona primers and therefore specific primers for each strain have to be used [65]. There is no specific anti-viral therapy available against HCoV-HKU1.

16.2.6 Human Bocavirus (2005)

Human bocavirus (HBoV) belongs to the genus *Bocavirus* within the family *Parvoviridae* (and is closely related to the bovine parvovirus and canine minute virus). This virus was identified in 2005 by nucleic acid amplification (PCR) in respiratory tract specimens from Swedish children with lower respiratory tract infections [66]. In this study HBoV was detected in 3.1 % of hos-

pitalized children below the age of three. Other studies detected HBoV in 3–19 % of children with respiratory symptoms depending on the sample type used (NPA and BAL *higher*, nasal swab *lower*) [67] and the age of the patient (*higher* in younger children) [68]. However, HBoV is frequently found in asymptomatic children (up to 40 %) or in combination with other viruses (up to 80 %) in symptomatic children [39]. Based on these findings it is still unclear whether HBoV has a pathogenic role in respiratory disease. One study performed in a PICU suggests that the viral load (high titres) of HBoV may indicate a pathogenic role in (severe) respiratory disease [69]. HBoV has been associated with wheezing in asthmatic children [70]. In general HBoV infection is marked by relatively mild symptoms of the upper respiratory tract such as cough, rhinorrhea and fever. In rare cases it has been associated with lower respiratory tract infection and even respiratory insufficiency [71]. Detection of HBoV is by RT-PCR and the virus can be detected in respiratory as well as gastrointestinal specimens [72]. Diagnostic seroresponses can be used to establish the specific immune response against HBoV during infection, although the clinical relevance is unclear [73]. HBoV can only be cultured on ciliated primary human epithelial cell-lines, and therefore viral isolation is only used in experimental settings [74]. Treatment of HBoV infections is mainly supportive and no specific anti-viral treatment against HBoV is available. Currently there is not enough epidemiological evidence to drive vaccine development against HBoV.

16.2.7 Parechovirus (2006–2007)

Human parechoviruses (HPeVs) belong to the genus *Parechovirus* of the family *Picornaviridae*. The first HPeVs, serotype 1 and 2, were identified 50 years ago during a summer diarrhoea outbreak in American children [75]. With the introduction of molecular techniques many new serotypes of HPeVs have been identified in the past 15 years in the stool or NPA of children with gastrointestinal and respiratory disease, and in the cerebrospinal

Table 16.4 HPeV, discovery and clinical associations

HPeV type	Known since	Clinical associations
HPeV1	1956	Mild gastrointestinal and respiratory symptoms, bronchiolitis, pneumonitis, otitis media. Encephalitis, paralysis, myocarditis
HPeV2	1956	Mild gastrointestinal and respiratory symptoms, (rare) neonatal sepsis, meningitis, encephalitis
HpeV3	2004	Neonatal sepsis, meningitis, encephalitis (transient paralysis)
HPeV4	2006	Fever, mild gastrointestinal and respiratory symptoms
HPeV5	2006	Fever, mild gastrointestinal and respiratory symptoms (sepsis, Reye's syndrome)
HPeV6	2007	Fever, mild gastrointestinal and respiratory symptoms (paralysis, Reye's syndrome)
HPeV8	2009	Enteritis
HPeV10	2010	Gastro-enteritis

fluid of children with meningitis and sepsis-like illness (see Table 16.4) [76–79]. Every HPeV serotype has its specific epidemiological and clinical features. All HPeVs infections are very common in children under the age of 1 year and most data are available on HPeV1 and HPeV3 [80]. The median age of infection with HPeV1 is 6.6 months, whereas HPeV3 infections occur at a younger age of 1.3 months. There is also seasonal variability in occurrence, HPeV1 in late summer and early winter season, and HBeV3 mostly in summer. HBeV serotype 5 and 6 have also been associated with respiratory tract symptoms [81–84].

Most HPeVs have are common causes of asymptomatic infection in early childhood and are often found in combination with other viruses, so that the relation with respiratory disease is hard to establish [80]. While the association of HPeV3 with encephalitis, meningitis and neonatal sepsis is widely accepted [85], for most other serotypes the relationship with disease and specific symptoms is less clear (see Table 16.4) [86].

A viral neutralisation assay or culture are time-consuming and not suitable for severe disease such as sepsis and meningitis. Detection by RT-PCR is only available for HPeV1-3 [87]. Currently amplification and nucleotide sequencing is used to identify specific genotypes in a research setting. The specific antibody response can be used to demonstrate involvement of HPeV in disease if the virus itself cannot be detected. No antiviral treatment against parechoviruses is currently available and only supportive care is given.

16.2.8 Polyomavirus (2007)

In 2007 two new members of the *Polyomaviridae* family were discovered in samples of patients with respiratory disease. The first of these new polyomaviruses was identified during a large scale molecular virus screening project in respiratory samples from children and named after the Karolisnka institute where it was discovered (KIV) [88]. The second was identified in a nasopharyngeal aspirate of a 3-year-old child with pneumonia and named Washington University virus (WUV) [89]. Seroprevalence studies show KI in 66 % and WU in 79 % of paediatric sera [46, 90]. The virus has been detected in 1–5 % respiratory samples worldwide in respiratory samples of young symptomatic children [91–93]. However, in 70–80 % of the cases there was a co-infection with other respiratory viruses, and KIV and WUV have been described in asymptomatic HSCT patients [94]. Based on these results it is difficult to assign symptoms and pathogenicity to both of them and more epidemiological evidence is needed. In most studies the viruses have been associated with both upper and lower respiratory tract infections in children. Detection of WUV and KIV in respiratory samples can be undertaken by RT-PCR. Thus far there is no indication for treatment of either of these viruses nor vaccine development.

16.2.9 Influenza A H1N1 Virus (2009)

In late march 2009 a novel influenza A (H1N1) virus was identified in America. This virus was subsequently recognised as the cause of an outbreak of respiratory illness in Mexico [95]. The

Table 16.5 Symptoms of H1N1

Presentation	Literature[a] (%)
Fever (>38 °C)	81–94
Cough	69–82
Gastro-intestinal symptoms	8–32
Rhinorrhea	31–62
Diarrhea	8–23
Wheezing	12–25

[a] Libster et al. [97], Hackett et al. [128], Jain et al. [129]

novel flu virus showed reassortment of swine, avian and human strains, and appeared to be very infectious between humans [96]. After the initial detection several other countries reported H1N1 infections. In June 2009 the WHO declared a pandemic with spread over at least two continents. At the start of the pandemic the virus appeared to be very virulent with a high mortality rate, especially in young adults and children [97, 98]. However, in the Northern Hemisphere the virus behaved more like a seasonal influenza virus. H1N1 disease had the highest attack rate in young children causing relatively mild disease [99]. The pH1N1 was able to outcompete the seasonal flu so that, in the influenza season 2009–2010, over 99 % of the influenza positive isolates in Europe and America were pandemic H1N1 influenza A [101].

In general the symptoms resembled those of other winter viruses: fever, cough, sore throat, myalgia and headache. Symptoms at presentation for hospitalised patients are shown in Table 16.5. Spread occurs up to 8 days after the start of symptoms although this may be prolonged in immunocompromised patients and children [102].

H1N1 infection can be diagnosed by RT-PCR on respiratory samples and this appears to be the most sensitive method. In case of high suspicion of H1N1 infection with a negative PCR result, the virus can be cultured or infection proven by documenting seroconversion [103–105].

During the pandemic of H1N1 were treated with oseltamivir (Tamiflu®) and zanamivir. This treatment reduced the duration of symptoms, the occurrence of otitis media and progression into

severe disease, especially when administered early in the course of disease [106]. Also the prophylactic use of anti viral agents is effective in reducing the occurrence of H1N1 infections in exposed individuals. However, oseltamivir and multi drug resistant viruses are emerging [107]. In several countries children have been vaccinated [108, 109]. H1N1 vaccination induced an effective and long lasting humoral immune response [108, 109]. The vaccine seemed to reduce the risk of infection and decreased severity of disease in children, however because of the rapid spread of the H1N1 pandemic most people were vaccinated during the pandemic making efficacy studies complex [111, 112].

16.3 Discussion on Molecular Diagnostics of Respiratory Viruses and Their Clinical Use

In this review we have discussed newly identified and emerging viruses from the past 2 decades. These viruses could be subdivided in three categories, based on the evidence for their pathogenicity in respiratory disease in children. First, emerging viruses causing epidemics with high mortality, such as AIV, Hendra and Nipah virus, were clearly associated with a pathogenic role in disease. These epidemic-causing viruses are often of zoonotic origin (transmission from animals to humans). The second group comprises viruses that fulfil the modified Koch's postulates [8, 9]. Most novel respiratory viruses are not completely characterised according to the postulates due to the extensive and costly research needed to achieve this. In this perspective hMPV is unique among the recently discovered respiratory viruses, because all criteria have been fulfilled [111]. Third are viruses that were found during screening for new respiratory viruses in respiratory samples with molecular techniques, such as human bocavirus, the novel polyomaviruses, parechoviruses and some coronaviruses. For most of these viruses their pathogenic role as an important respiratory pathogen is less clear. Although these viruses are present in respiratory

samples of children with respiratory disease however, they are also often present in asymptomatic children or found in combination with other viruses. Many studies were performed retrospectively, or without the proper control cohorts of asymptomatic children. In epidemiological studies based on seroconversion it is apparent that a first encounter with these viruses occurs early in childhood without (severe) respiratory tract infections. Especially in this last category of viruses, in which the association with respiratory disease is less clear, large prospective epidemiological studies are needed to further specify the pathogenicity and health burden of these viruses in children.

The highly sensitive molecular techniques for identification and detection of novel viruses are a powerful tool for epidemiological studies, especially when used in multiplex platforms. Their ability to quantify the viral burden in infection may be used as additional information in determining the role of a virus in respiratory disease. For some viruses a positive correlation between viral load and disease severity is described [69, 115]. However whether viral load correlates with disease severity in general remains a point of debate. Viral load appears to be lower in viral-viral co-infection compared to viral-mono-infections, the mechanism behind this and the clinical relevance requries further investigation [116, 117]. Studies show that viral load decreases during the course of disease, and this can be used as marker for the therapeutic effect of anti-viral compounds. A drawback of the high sensitivity of molecular diagnostics is that PCR signals remain positive after recovery from an illness, sometimes even for several weeks. Because young children have frequent viral infections of the upper respiratory tract, the value of a positive PCR test can be limited.

Interaction of viruses with bacteria present in the nasopharynx can result in enhanced disease severity. This is well known for influenza and *Streptococcus pneumoniae*, and other respiratory bacteria [118]. How other (novel) respiratory viruses interact with bacteria and how this leads to enhanced disease is less well known. In study-ing the pathogenicity of viruses these interactions should be taken into account and implemented in new epidemiological studies. In this context 'old' viruses, like rhinovirus, can be seen in a new perspective and the causality with severe respiratory disease should be re-evaluated [118–120].

The introduction of molecular detection of viruses has led to the discovery of many new human respiratory viruses and improvement in diagnostics. Novel molecular techniques, like sequencing of the complete virome, will offer new insight in viral infections but also new challenges in proving causality in human disease.

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Urinary Tract Infections in Children: Microbial Virulence Versus Host Susceptibility

Catharina Svanborg

Abstract

Urinary tract infections (UTI) are common, dangerous and interesting. This review includes a general background on UTIs and molecular mechanisms of pathogenesis. In addition, we discuss UTI susceptibility and especially the effect of genetic variation on innate immunity.

The symptoms of acute pyelonephritis are caused by the innate immune response and inflammation in the urinary tract decreases renal tubular function and may give rise to renal scarring, especially in childhood. The disease severity is explained by pathogens and their virulence factors triggering signaling through Toll-like receptors (TLRs), interferon regulatory factors (IRFs) and type 1 interferons, and the activation of a host response mediating disease or pathology or clearance of infection. In children with asymptomatic bacteriuria (ABU), in contrast, bacteria persist without causing symptoms or pathology. ABU strains mostly lack virulence factors, and the lack of symptoms has largely been attributed to their lack of virulence. Recently, rapid progress has been made in the understanding of host susceptibility mechanisms. For example, genetic alterations that reduce TLR4 function are associated with ABU while polymorphisms reducing IRF3 or CXCR1 expression are associated with acute pyelonephritis and an increased risk for renal scarring.

Understanding bacterial virulence and host resistance promises new tools to improve the diagnostic accuracy in children with UTI. By combining information on bacterial virulence and the host response, it should be possible to start individualizing diagnosis and therapy. Finally, we propose that the prediction of future disease risk and decisions on prophylaxis and invasive diagnostic procedures might be improved by genetic analysis.

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17.1   Introduction

Bacteria frequently invade the distal urinary tract but in most individuals, infection is not established, due to an efficient antibacterial host defense, reviewed below. However, in patients with voiding dysfunction, malformations and/or molecular immune defects, infection is facilitated, and even bacteria of reduced virulence may establish infection. Most children who develop UTI have a normal urinary tract, and to establish bacteriuria and symptomatic infection, specific virulence factors are required as well as a match with a susceptible host.

Urinary tract infection (UTI) is defined by significant bacteriuria, classically $>10^5$ CFU/ml of urine, but lower counts are considered relevant in a patient with acute symptoms and frequent voiding. Bacteriuria may lead to severe symptoms and even long-term sequelae such as renal scarring, or be asymptomatic and protect against symptomatic disease.

Acute pyelonephritis (APN) is the most severe form of UTI, accompanied by local and systemic inflammation and symptoms. Bacteria establish infection in the renal pelvis and may proceed to invade renal tissues. The patient experiences flank pain, fever >38.5 and general malaise and laboratory tests detect elevated levels of circulating acute phase reactants like CRP, ESR, cytokines and leucocytes. Increasingly used to also quantify local inflammation in the urinary tract are urine cytokine levels, adding much-needed information in addition to pyuria, the classical diagnostic tool to detect inflammation in the urinary tract. Adults with acute pyelonephritis develop bacteremia in about 30 % of cases and urosepsis remains an important cause of death. The frequency of bacteremia or uro-sepsis in children is lower, and, in the pediatric age group, it is not entirely clear to what extent bacteria invade the blood-stream.

Asymptomatic bacteriuria (ABU) is the most common form of UTI, occurring in about 1 % of girls, 2 % of pregnant women and 20 % of both men and women above 70 years of age. These patients carry $>10^5$ CFU/ml of urine, often for extended periods of time, without developing symptoms. The patients may develop a mild host response that can be quantified as low cytokine levels in urine (IL-8 but not IL-6) as well as variable, low level pyuria and after long-term carriage the patients may develop lymphoid follicles in the bladder mucosa, which resolve after antibiotic therapy. This host response is obviously not strong enough to cause symptoms and the relevance of variable host responses in ABU is unclear.

Acute cystitis is very common, but not well defined in childhood, as diagnosis largely relies on the patient being able to describe the symptoms. In adult women, acute cystitis episodes are exceedingly common, with frequency, dysuria and suprapubic pain and often require antibiotic therapy. In addition, lower tract symptoms may precede upper tract infection, creating a grey zone between patient groups with symptomatic UTI.

The level and severity of UTI is determined by the properties of the infecting strain; mostly *Escherichia coli*. Virulent uropathogenic *E. coli* that cause acute pyelonephritis are carried for extended periods of time in the fecal/perineal flora of highly UTI prone individuals. Invasion of the urinary tract is hindered by an intact urine flow and a highly efficient innate immune response, which senses the presence of invaders and destroys them, so that the urinary tract can regain its "sterility".

UTIs are also a fascinating model, to study the molecular features of host-pathogen interactions at mucosal surfaces. Unlike other mucosal sites that are populated by a complex bacterial flora, the urinary tract is usually infected by a single strain, making analyses of virulence factors and their contribution to human disease quite feasible. Host response variables may also be assessed and directly correlated to disease severity. This chapter focuses on determinants of bacterial virulence and on the host response, especially dysfunctions that tilt the balance towards inflammation, symptoms and disease.

17.2   Pathogenesis of UTI

E. coli strains cause 80–90 % of community-acquired acute pyelonephritis episodes and are especially common in children. Uropathogenic *E. coli* (UPEC) strains are defined by chromosomal virulence genes, which may be activated at different times during infection, to facilitate host tissue attack and bacterial survival. For example, bacterial adhesion to the mucosa is a crucial first step in disease induction, and fimbriae increase tissue exposure to secreted bacterial toxins, like hemolysin and LPS, that damage the tissues by interfering with cellular functions or by directly inducing cell death. UPEC strains can be identified, by typing for virulence factors like P fimbriae.

Critical mechanisms involved in antibacterial host defense have also been identified. Resistance to infection relies on innate immune mechanisms and defects in innate immunity drastically increase UTI susceptibility, both in mice carrying single gene deletions and in patients prone to acute pyelonephritis or ABU. In contrast, specific immunity executed by T lymphocytes and/or B lymphocytes is not involved in acute defense. These findings are consistent with clinical observations, showing that susceptibility to acute pyelonephritis is not associated with hypo-gammaglobulinemia or T lymphocyte deficiencies.

17.3   Bacterial Virulence Factors

17.3.1   Bacterial Adhesion

The ability to adhere to cells in the urinary tract is a critical virulence factor in UTI. UPEC trains express surface fimbriae that recognize specific receptors on host cells. The different fimbrial types are distinguished by their structural features and by the oligosaccharide receptor epitopes that they recognize. Type l fimbriae bind to mannosylated glycoproteins, such as the Tamm-Horsfall glycoprotein, secretory IgA or bladder cell uroplakin. S fimbriae bind sialic acid epitopes present in sialylated glycoproteins and glycolipids. P-fimbriae recognize Galα1-4Galβ-epitopes in the globoseries of glycolipids. The expression of P fimbriae is strongly linked to virulence, being present in about 80 % of strains causing uncomplicated acute pyelonephritis but in <20 % of strains causing ABU. The frequency of P fimbriated strains is reduced in patients with impaired resistance, due to pregnancy, urodynamic defects or genetic abnormalities (see below).

17.3.2   Lipopolysaccharide (LPS)

LPS is the endotoxin of Gram-negative bacteria, containing the membrane anchor Lipid A which is responsible for the toxic effects. Injection of Lipid A mimics many of the symptoms associated with acute pyelonephritis, Gram negative septicemia or other serious Gramnegative infections, including fever, and activates the acute phase response. In addition, LPS carries an invariable oligosaccharide core and a repeating oligosaccharide that determines the O antigen. LPS activates Toll-like receptor 4 (TLR4) signaling, after binding to LBP, CD14 and MD2. This mechanism is essential for innate immune activation during systemic infection but is less important at mucosal surfaces, where the cell-bound CD14 receptor often is missing and MD2 may be weakly expressed.

17.3.3   Capsular Polysaccharide

Capsular polysaccharides are formed from highly repetitive, oligosaccharides and different capsular types are defined by variant antigenic oligosaccharide epitopes. The capsules surround bacteria in blood and tissues and contribute to bacterial survival in the presence of a functional host defense, by preventing lytic effects of complement and antibacterial peptides as well as phagocytosis. Mutant bacteria with altered capsule expression show reduced virulence in experimental UTI models.

17.3.4 Hemolysin

Hemolysins are cytotoxic, pore-forming proteins that permeate the cell membrane, commonly identified by their ability to lyse erythrocytes. Hemolysin production was observed in *E. coli* causing acute pyelonephritis in the 1940s, and hemolysin occurs more often in *E. coli* causing acute pyelonephritis than in fecal isolates even though the difference in frequency is less pronounced than for e.g. P fimbriae.

17.3.5 Metabolic Competition

Most *E. coli* strains causing acute pyelonephritis produce aerobactin, which is an iron binding protein with high affinity, as well as other iron sequestering proteins. By producing such proteins, bacteria compete with the host for nutrients such as iron. Host iron-binding proteins include lactoferrin in mucosal secretions and transferrin in the circulation, and their concentrations increase in response to infection.

17.4 The Innate Immune Response

The innate immune response is activated in a pathogen specific way by P fimbriae mediated adhesion to glycolipid receptors, followed by release of ceramide, activation of TLR4 signaling, and downstream transcription factors like IRF3, which trigger cytokine production and neutrophil recruitment, eventually killing the bacteria. Indeed, the presence of a mucosal cytokine response was first observed in UTI. IL-6 is secreted by uroepithelial cells and acts as an endogenous pyrogen, activates hepatocyte CRP production and stimulates mucosal B cells to produce IgA antibodies. The biological activities of IL-6 thus agree with the clinical presentation of acute pyelonephritis and IL-6 is detected in the blood and urine of patients with acute pyelonephritis.

Infected epithelial cells also secrete IL-8, which is chemotactic for neutrophils. IL-8 forms a gradient across the mucosa and neutrophils migrate towards the gradient, and cross the mucosal barrier and into the urine. The concentrations of IL-8 correlate with the number of leucocytes in urine, and the chemotactic activity of urine is inhibited by IL-8 antibodies, showing that IL-8 is an essential chemoattractant in UTI. In addition, infection increases the expression of IL-8 receptors, which are important targets for the IL-8 family of chemokines and support neutrophil migration and activation. These mechanisms explain "pyuria", i. e. the presence of leucocytes in urine, which has been used as a diagnostic tool in UTI since microscopy was first used in the clinic. More recently, a number of additional chemokines have been found in urine, probably reflecting a more complex immune response in the mucosa, especially during long-term bacterial carriage.

These mechanisms also determine the symptoms and signs of acute UTI and several studies have suggested that urine cytokine levels directly reflect the degree of renal involvement.

17.5 Host Factors that Predispose to UTI

The frequency of UTI varies with age, gender and socio-economic factors. UTI morbidity is higher in women than in men, except during the first year and at the end of life. Regardless of gender, certain individuals develop severe, acute infections, some recur and a subset develop a chronic state of tissue damage, leading to renal scarring. For decades, scientists have worked to better understand the factors that determine susceptibility and to develop diagnostic tools to discriminate these individuals from others, who only experience sporadic UTI episodes. Recent studies have started to identify genetic risk factors that distinguish pyelonephritis-prone children from those who consistently develop ABU. Even if the information is limited, it is clear that genetic variation affecting innate immunity influences UTI susceptibility.

Resistance to acute pyelonephritis is also influenced by dysfunctional voiding. Patients with reflux are susceptible to infection with bacteria of lower virulence than patients with anatomically normal urinary tracts. It is not clear if the urine flow, *per se*, is sufficient to maintain

sterility, aided by antibacterial peptides that directly kill the bacteria.

17.5.1   Inflammation and Bacterial Clearance

The inflammatory response is crucial for the antibacterial defense of the urinary tract. Loss of functional Toll-like receptors impairs the innate immune response, and promotes long-term bacterial colonization and ABU. In contrast, defects in the antibacterial effector functions encoded by this pathway, result in severe acute infections, tissue damage and renal scarring.

17.5.2   Toll-like Receptor Signaling and ABU

Children with ABU express lower levels of the TLR4 receptor than age matched controls or children with acute pyelonephritis. They carry mutations in the TLR4 promoter that reduce the efficiency of TLR4 expression. Mice lacking the TLR4 gene also develop ABU, with $>10^5$ CFU/ml of bacteria but no symptoms or tissue damage. Low TLR4 expression and the resulting absence of inflammation can thus be protective rather than destructive.

17.5.3   IRF3, CXCR1 and Acute Pelonephritis

The IRF3 transcription factor enhances the transcription of innate immune response genes including cytokines and chemokine receptors. Mice lacking *Irf3* develop severe acute pyelonephritis with urosepsis and renal abscesses. Mice lacking the IL-8 receptor ($mCxcr2^{-/-}$) also develop severe acute pyelonephritis with urosepsis and surviving animals have renal abscesses and renal scarring. These examples show that single gene deletions affect crucial functions in innate immune signaling, resulting in increased UTI susceptibility in mice. The pathology of the $mCxcr2^{-/-}$ was also due to poor neutrophil recruitment and neutropil activation. Like the $Irf3^{-/-}$ mice, the $mCxcr2^{-/-}$ mice showed acute renal abscesses and surviving mice developed renal scarring.

We also found *IRF3* and *CXCR1* polymorphisms in pyelonephritis prone patients. Children prone to acute pyelonephritis had reduced CXCR1 expression and heterozygous CXCR1 polymorphisms and low mRNA levels suggested that CXCR1 transcription is impaired in this patient group. We also found promoter polymorphisms that reduced promoter efficiency and IRF3 expression in pyelonephritis prone children.

17.5.4   Receptors, Blood Groups and UTI Susceptibility

Uropathogenic *E. coli* colonize mucosal surfaces through adhesion, and thus, resistance to infection can be modified by the repertoire of receptors that the patients express and by soluble, anti-adhesive molecules. Early studies by Stamey et al. proposed that women with recurrent UTI have increased vaginal carriage of uropathogenic bacteria and their uroepithelial cells have since been shown by several groups to have an increased tendency to bind uropathogenic *E. coli,* reflecting an increased density of receptors.

P fimbriated *E. coli* bind to the globoseries of glycolipids, which are antigens in the P blood group system. Through P blood group determination, it is possible to predict the mucosal receptor repertoire and thus, the P blood group can be used as a marker in epidemiologic studies. Individuals of blood group P lack a critical enzyme involved in glycolipid synthesis and lack functional receptors for P fimbriae predicting that they would be more resistant to infection with P fimbriated bacteria. Children of blood group P1 run an increased risk of acute pyelonephritis (about 11 fold) and carry P fimbriated *E. coli* in the intestinal flora, which is a UTI risk factor. Epithelial receptor expression is also influenced by the ABO blood group and individuals expressing globo A are preferentially infected by P fimbriated *E. coli* that recognize a combined Galα1-4Galβ and blood group A epitope.

17.5.5 Anti-adhesive Molecules in Urine

Urine contains soluble receptor oligosaccharides or glycoconjugates that bind fimbriae and prevent them from mediating adhesion. The Tamm-Horsfall protein, which is a normal urine constituent, carries terminal mannose residues, that bind type 1 fimbriated *E. coli* and sialic acid residues bind S fimbriae. P fimbriae, which show the strongest disease association, have no known soluble inhibitors in urine.

17.5.6 Specific Immunity and Resistance to UTI

Acute pyelonephritis gives rise to a specific immune response after three to seven days. The mucosal immune response has a relatively short memory, however, and thus most patients have low levels of specific antibodies in urine, when they develop recurrent infections. In experimental studies, mice lacking a specific immune system have surprisingly not shown a drastic increase in UTI susceptibility, and patients with different specific immuno-deficiencies do not primarily develop UTI, suggesting that the specific immune system is not essential for the defense against acute infection.

The main evidence that specific immunity may protect the urinary tract against infection has been generated through vaccination studies. Early studies showed that vaccination with formalin treated whole bacteria markedly increases specific antibody levels. Further studies have used purified antigens (LPS, kapsel, fimbriae, outer membrane proteins) and show that hyper-immunization can protect against infection, provided that the vaccine antigen is expressed by the bacterial strain used for infection and infectious challenge is given while the immune response remains active. The antigenic variation in uropathogenic *E. coli* is a problem for the implementation of vaccination in UTI, however, modern vaccine development gives rise to new optimism. The genetic studies also suggest that genetically susceptible individuals might be targeted for vaccination.

17.6 Summary

The symptoms of acute pyelonephritis are caused by the innate immune response and urinary tract inflammation decreases renal tubular function and may give rise to renal scarring, especially in children. Antibacterial defence relies on innate immunity and TLR4 signaling initiates this response. Reduced TLR4 function is associated with ABU, while reduced IRF3 or CXCR1 expression is associated with severe acute pyelonephritis and an increased risk of renal scarring.

Most cases of acute pyelonephritis are caused by *E. coli* strains expressing virulence factors that contribute to the establishment of bacteriuria. P fimbriae remain the single virulence factor that best describes the virulence of the infecting strain, but more extensive gene expression methods may also be used to get a more complete picture of the virulence repertoire.

The characterization of bacterial virulence factors and inflammatory profiles may provide new tools for the diagnosis of UTI. Virulent bacteria can, for example, be identified by the expression of P fimbriae. By combining information on bacterial virulence and the host response, it should be possible to start "individualizing" diagnosis and therapy in the large and diverse group of patients with UTI.

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Preventing Urinary Tract Infections in Early Childhood

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Abstract

Urinary tract infection (UTI) is common in children, causes them considerable discomfort, as well as distress to parents and has a tendency to recur. Approximately 20 % of those children who experience one infection will have a repeat episode. Since 1975, 11 trials of long-term antibiotics compared with placebo or no treatment in 1,550 children have been published. Results have been heterogeneous, but the largest trial demonstrated a small reduction (6 % absolute risk reduction, risk ratio 0.65) in the risk of repeat symptomatic UTI over 12 months of treatment. This effect was consistent across sub groups of children based upon age, gender, vesicoureteric reflux status and number of prior infections. Trials involving re-implantation surgery (and antibiotics compared with antibiotics alone) for the sub-group of children with vesicoureteric reflux have not shown a reduction in repeat UTI, with the possible exception of a very small benefit for febrile UTI. Systematic reviews have shown that circumcision reduces the risk of repeat infection but 111 circumcisions would need to be performed to prevent one UTI in unpredisposed boys. Given the need for anaesthesia and the risk of surgical complication, net clinical benefit is probably restricted to those who are predisposed (such as those with recurrent infection). Many small trials in complementary therapies have been published and many suggest some benefit, however inclusion of children is limited. Only three trials involving 394 children for cranberry products, two trials with a total of 252 children for probiotics and one trial with 24 children for vitamin A are published. Estimates of efficacy vary

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widely and imprecision is evident. Multiple interventions to prevent UTI in children exist. Of those, long-term low dose antibiotics has the strongest evidence base, but the benefit is small. Circumcision in boys reduces the risk substantially, but should be restricted to those at risk. There is little evidence of benefit of re-implantation alone, and the benefit of this procedure over antibiotics alone is very small. Cranberry concentrate is probably effective.

18.1 Background

18.1.1 Frequency

Urinary tract infection (UTI) is a very common illness in children, affecting 2 % of boys and 8 % of girls by the age of 7 years [1]. It is also the most common serious bacterial infection in children with fever who present for assessment [2, 3] and causes an unpleasant acute illness with manifestations that include fever, lethargy, vomiting and cystitis symptoms.

18.1.2 Recurrence

Good evidence to quantify the risk of recurrence and identify factors that may predispose to repeat infections is quite scarce. Studies that follow children with UTI over time are required, and there have been only a few. Two studies of this design [4, 5] have demonstrated that about 12 % of children with first UTI experience a recurrence within one year. The placebo arm of a large, blinded trial [6] showed a recurrence rate of 19 % in 12 months, but eligibility criteria were not limited to the first infection, so this may be an over-estimate of the true risk.

18.1.3 Risk Factors for Recurrence

Some children are more at risk for future UTIs than others. Risk factors include an age less than six months at first UTI, grade III–IV vesicoureteric reflux and white race. Early observations

that UTI and vesicoureteric reflux were associated with renal damage [7–9] led to the standard practice of performing voiding cystourethrography to identify reflux in children with a history of UTI [10, 11].

18.2 Antibiotic Treatment

Children with reflux were routinely given daily low-dose antibiotics for many years [12] with the aim of preventing further UTI and renal damage. Until 1997, only four trials [13–16] with 171 children and conflicting findings provided the evidence base for this treatment (Table 18.1). Since then seven trials [6, 17–22] have been published with broader criteria, including children with reflux and designs which are less prone to bias. Six of the seven trials showed a reduced risk of repeat symptomatic UTI with prophylactic antibiotics but the magnitude of the effect was small and in most studies the difference did not reach statistical significance (Fig. 18.1). In the largest ($n = 576$) and importantly the only blinded study [6], the benefit was statistically significant, and showed a 6 % absolute risk reduction for repeat symptomatic UTI in children taking trimethoprim sulphamethoxazole for 12 months. Benefits did not vary according to baseline characteristics such as age, gender and reflux status in *a priori* sub-group analysis. Five trials [6, 18, 20–22] also reported rates of bacterial resistance to the prophylactic drug and all showed substantial increases. Overall, there appears to be a small benefit from prophylactic antibiotics (6 % absolute risk reduction, or an overall number needed to treat of 16 over 1 year) but this must be weighed against the proven risk of increased bacterial resistance to antibiotic and with consideration for suggested but uncertain effects such as susceptibility to asthma and inflammatory bowel disease [23, 24].

Five trials have compared one antibiotic with another [25–29], two of which compared cotrimoxazole with nitrofurantoin [26, 29]. These two trials were small (N of 120 and 132) but both demonstrated statistically significant superiority of nitrofurantoin (risk ratios (RR) 0.57 (95 % CI
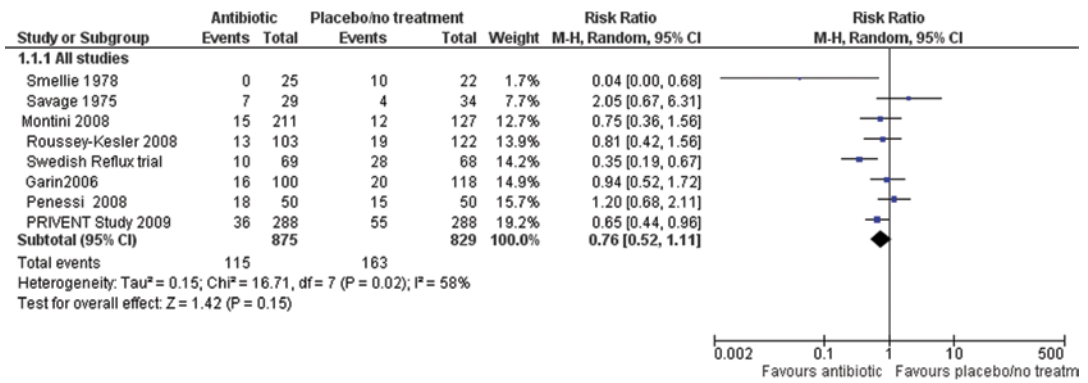
Study or Subgroup	Antibiotic Events	Total	Placebo/no treatment Events	Total	Weight	Risk Ratio M-H, Random, 95% CI	Risk Ratio M-H, Random, 95% CI
1.1.1 All studies							
Smellie 1978	0	25	10	22	1.7%	0.04 [0.00, 0.68]	
Savage 1975	7	29	4	34	7.7%	2.05 [0.67, 6.31]	
Montini 2008	15	211	12	127	12.7%	0.75 [0.36, 1.56]	
Roussey-Kesler 2008	13	103	19	122	13.9%	0.81 [0.42, 1.56]	
Swedish Reflux trial	10	69	28	68	14.2%	0.35 [0.19, 0.67]	
Garin2006	16	100	20	118	14.9%	0.94 [0.52, 1.72]	
Penessi 2008	18	50	15	50	15.7%	1.20 [0.68, 2.11]	
PRIVENT Study 2009	36	288	55	288	19.2%	0.65 [0.44, 0.96]	
Subtotal (95% CI)		875		829	100.0%	0.76 [0.52, 1.11]	
Total events	115		163				

Heterogeneity: Tau² = 0.15; Chi² = 16.71, df = 7 (P = 0.02); I² = 58%
Test for overall effect: Z = 1.42 (P = 0.15)

0.002   0.1   1   10   500
Favours antibiotic   Favours placebo/no treatm

Fig. 18.1 Randomised controlled trials of antibiotic compared with placebo/no treatment for the prevention of repeat symptomatic UTI in children

0.35–0.92) and 0.32 (95 % CI 0.19–0.56)). However, one study [26] reported 30 % of patients withdrawing from the study due to side effects of nitrofurantoin suggesting the acceptability of this treatment may be poor.

18.3   Surgical Treatment

Surgical correction of the physical abnormality of reflux can involve open surgery to reimplant the ureters or endoscopic injection of agents at the vesicoureteric junction. A recent systematic review of these treatments [30] showed no convincing evidence of a reduced risk of repeat symptomatic UTI at 1–2, 4–5 or 10 years after surgery (and antibiotics) compared with antibiotics alone (1–2 years RR 0.88 (95 % CI 0.26–3.01), 5–10 years RR 0.79 (95 % CI 0.49–1.26), 10 years RR 1.06 (95 % CI 0.78–1.44)). These trials and the numerous case series found in the literature usually demonstrate a very high rate of surgical correction of reflux in treated children but without matching reduction in risk of UTI, suggesting that reflux only has a modest attributable risk for further UTI.

18.3.1   Circumcision

A systematic review of trials and observational studies of circumcision [31] to prevent UTI showed that circumcision reduces the risk of UTI but that 111 circumcisions would need to be performed to prevent one UTI in normal boys with a baseline risk of 1 %. Major complications occur in approximately 2 %. Circumcision could be considered in boys predisposed to UTI to achieve net clinical benefit, such as those with recurrent infection and/or those with high grade reflux. In this scenario the number needed to treat would be about 5–10.

18.4   Complementary Treatments

Systematic reviews and trials of complementary treatments for prevention of UTI have been conducted but most do not include a substantial number of children. A systematic review of cranberry products [32] suggests some benefit in preventing recurrent UTI in women but insufficient data were available for conclusions about efficacy in children. Since that review three trials in children have been published [33–35], two only in abstract form [34, 35], and all demonstrated apparent benefit in cranberry product use but only one reached statistical significance [33]. The smallest study ($n = 51$) compared cranberry with antibiotic treatment trimethoprim and suggested that cranberry may be more effective (relative risk of 0.65 (95 % CI 0.34–1.25) while the larger, blinded study ($n = 263$) gave a less favourable relative risk of 0.74 (95 % CI 0.44–1.25). Neither

Table 18.1 Randomised controlled trials for interventions to prevent urinary tract infection in children

First author	Year	Number	Participants			Intervention			Comparator	Blinded	Event rate (%)	Outcomes			Results
			Age (years)	N VUR	VUR grades	NF	TMP-SMZ	Other	Placebo			Symptomatic UTI	Positive culture	Abn DMSA	Risk ratio (95 % CI) symptomatic UTI, (positive culture)
Low dose, antibiotic therapy compared with placebo/no treatment															
Savage	1975	63	5–7.8	16	I–III	•	•		x	?	7/63 (11.1)	•	•		2.05 (0.67, 6.31) 0.93 (0.74, 1.17)
Stansfield	1975	45	0.5–14	13	NS		•		✓	✓	12/45 (26.7)		•		0.05 (0.00, 0.72)
Lohr	1977	18#	3–13	0	NA	•	•		✓	✓	15/18 (83.3)		•		0.13 (0.04, 0.50)
Smellie	1978	45	2–12	0	NA	•	•		x	x	11/47 (23.4)	•	•		0.04 (0.0, 0.68) 0.04 (0.0, 0.52)
Reddy	1997	43	NS	43	?	?	?	?	x	?	6/29 (20.7)	•	•	•	0.25 (0.03, 1.85)
Garin	2006	218	0.25–17	113	I–III	•	•		x	?	36/218 (16.5)	•	•	•	0.94 (0.52, 1.72) 0.74 (0.43, 1.28)
Montini	2008	338	0.08–8.4	128	I–III	•	•	•	x	x	27/338 (3.6)	•	•	•	0.75 (0.36, 1.56) 0.50 (0.29, 0.86)
Roussey-Kesler	2008	225	0.08–3	225	I–III	•	•		x	?	32/225 (14.2)	•	•		0.81 (0.42, 1.56) 0.67 (0.40, 1.11)
Pennesi	2008	100	0–2.5	100	II–IV	•	•		x	x	33/100 (33.3)	•	•	•	1.20 (0.68, 2.11)
PRIVENT	2009	576	0–18	243	I–V	•	•		✓	✓	91/576 (15.8)	•	•	•	0.65 (0.44, 0.96)
Swedish Reflux Trial	2010	203	1–2	203	III–IV	•	•	•	x	x	49/203 (24.1)	•	•	•	0.39 (0.21 0.76)
Low dose, antibiotic A vs low dose antibiotic B															
Carlsen	1985	35#	1–13	17	I–III	•		Pivmecilliam	Alternative antibiotic	?	10/33 (30.3)		•		0.68 (0.28, 1.65)
Brentstrup	1990	130	1–14	51	NS	•	•		Alternative antibiotic	✓	31/120 (25.8)		•		0.32 (0.19, 0.56)
Lettgen	2002	57	1–11	NS	NS	•		Cefixim	Alternative antibiotic	?	5/57 (8.8)	•			1.35 (0.24, 7.48)

Table 18.1 (continued)

First author	Year	Number	Participants			Intervention	Comparator	Blinded	Event rate (%)	Outcomes	Results
Belet	2004	80	0.5–15	0	NA	Cephadroxil/cefprozil •	Alternative antibiotic	x	12/80 (15.0)	•	0.69 (0.20, 2.39) *1.35 (0.71, 2.56)* TMP_SMX vs cefprozil / 1.79 (0.33, 9.70) *5.95 (1.46, 24.21)* TMP_SMX vs cephadroxil / 0.39 (0.09 to 1.71) *0.23 (0.06, 0.92)* cephadroxil vs cefprozil
Falaflaki	2007	132	0.25–12	57	I–IV	• •	Alternative antibiotic	?	47/132 (35.6)	•	0.57 (0.35 to 0.92)
Alternative dose											
Baciulus	2003	33	0–16	8	?	Cefdroxil	Every vs alternate night dose	x	7/33 (21.2)	•	*1.11 (0.29, 4.21)*
Complementary therapies											
Salo^	2010	263	NS	35	?	Cranberry juice	Placebo juice	✓	48/263 (18.3)	•	0.74 (0.44, 1.25)
Uberos^	2010	51	NS	NS	NS	Cranberry syrup	Trimethoprim	✓	23/51 (45.1)	•	0.65 (0.34, 1.25) (cranberry vs trim)
Ferrara	2009	80	3–14	0	NA	Cranberry or probiotic	No treatment	x	34/80 (42.5)	•	0.28 (0.12, 0.64) cranberry vs no treatment / 0.63 (0.38, 1.07) probiotic vs no treatment / 0.44 (0.18, 1.09) cranberry vs probiotic
Lee^	2010	132	~0.08–0.8	132	?	Probiotic	TMP-SMX	?	47/128 (36.7)	•	0.81 (0.51, 1.28)
Lee	2007	120	1.08–3	120	I–V	Probiotic	TMP-SMX	?	24/120 (20.0)	•	0.85 (0.41 to 1.74)
Yilmaz	2007	24	>1–12	0	NA	Vitamin A (single dose)	Placebo	✓	?	•	RR not calculable, *Vit A 0.29/ mo vs 0.44/mo 6–12 mo*

Cross over trial
^ Published only in abstract form

study demonstrated statistical significance. This evidence suggests cranberry products may reduce the risk of repeat UTI in children but there is considerable uncertainty. None of these trials reported adverse events, however trials in adults suggest most adverse events are minor gastrointestinal issues.

A systematic review of methenamine hippurate [36] for preventing UTI concluded that the intervention may be effective in patients without renal tract abnormalities but no children were included in the trials. No new trials have been published to help resolve the uncertainty. A blinded, randomised placebo controlled trial of herbal products *Tropaeoli majoris* and *Armoraciae rusticanae* [37] in adults showed no difference in average number of recurrences between the two groups using intention to treat analysis. Two randomised trials of probiotics compared with cotrimoxazole treatment to prevent UTI in children with vesicoureteric reflux have been published [38, 39]. Neither study demonstrated a statistically significant difference but the point estimates favoured probiotics 0.85 (95 % CI 0.41–1.74) and 0.81 (95 % CI 0.51–1.28) [36, 37]. There remains uncertainty and imprecision about the efficacy of this intervention to prevent recurrent UTI in children.

A meta-analysis of trials [40] of the immune active agent, Uro-Vaxom showed it to be an effective treatment for preventing recurring UTIs, however none of the five included trials were large or optimally designed and each included only adults. Several treatment studies in children have been published [41, 42] but without randomisation nor a comparator these are not a firm basis for decision making.

A randomised, placebo controlled trial of vitamin A to prevent recurrent UTI in children [43] showed a reduced rate of UTI in the follow-up period, but only 24 children participated so the estimate of efficacy is imprecise and there are also concerns over selection bias.

18.5 Conclusion

Good quality evidence demonstrates that low dose antibiotics reduce the risk of repeat UTI in children by approximately 6 %, and is consistent across all groups of children. Clinicians now have a clear estimate of risk reduction along with details on adverse effects with which to discuss treatment options with parents. Given the relatively small absolute benefit, clinicians and families may opt for prophylactic antibiotics when the risk of recurrence is relatively high (e.g. in those with recurrent infection) or when the potential seriousness of an additional event is very significant (e.g. in a very young infant). Circumcision reduces the risk of repeat UTI but is best limited to those at higher risk of recurrence. Complementary therapies have been explored using randomised controlled trials and usually found to be effective but study design is generally poor and studies are small, leading to potential bias and imprecision. However, most of these interventions are usually free of adverse events and parents may elect to try them. They should be reminded to be alert to further infections and seek treatment when appropriate.

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How to Get and Get Rid of Gonorrhea

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Abstract

Gonorrhea remains as a significant public health concern with an estimated 88 million new cases per year globally. Gonorrhea is a disease of sexual networks and is most prevalent in youth, men who have sex with men, and the socioeconomically disadvantaged. Highly adaptive through years of co-evolution, gonorrhea has developed multiple ways of evading the human immune system. Although new molecular-based strategies have opened avenues for less invasive testing, education and accessibility issues persist. Novel strategies, including use of the internet and social media, are required to better target high risk groups for education, testing, and treatment. Increasing the availability of youth-friendly health services will also help foster earlier gonorrhea diagnosis and management. The inappropriate and overuse of antibiotics and propensity of gonococcus for mutation has led to growing microbe resistance. Treatment failures now include both oral and intravenous formulations of third generation cephalosporins; key front line recommended gonococcal treatment in many countries. With treatment options dwindling, the need for better preventative strategies has never been more important. This overview highlights some of the major aspects of gonococcal infection, including the epidemiology of the disease with an emphasis on sexual networks, new diagnostic techniques, treatment options in the face of evolving gonococcal resistance, and notes potential new preventative strategies.

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19.1 Introduction

Neisseria gonorrhoeae has been a sexually transmitted scourge of humans for eons. The relationship has been so long and so enduring that a piece of human DNA became entwined with the microbe's genome many millennia ago [1]. Unfortunately, gonorrhea continues to cause significant morbidity and mortality worldwide. A disease largely of the disadvantaged, gonorrhea disproportionately affects inhabitants of developing countries and those of lower social economic status, globally. Worldwide, the gonorrhea epidemic is primarily focused among young people due to a variety of social and behavioural factors shared by this group.

While little has changed in the clinical presentation of gonorrhea, our understanding of organism pathogenesis and the availability of diagnostic tools has grown. Additionally, multi-drug resistant gonococcal strains have now emerged. Without alternative antibiotics or a candidate vaccine in the pipeline, gonorrhea may soon become untreatable [2, 3]. With dwindling therapeutic options, new prevention strategies based on an understanding of sexual networks and utilizing youth-friendly information modalities, need to be pursued.

Rather than an exhaustive review, the purpose of this article is to supply a snapshot of gonorrhea in the world today with an emphasis on young people living in developed countries. Key topics explored include current epidemiology, risk factors for transmission, benefits and limitations of new diagnostic modalities, treatment options in the context of growing antimicrobial resistance, and novel preventative strategies.

19.2 Gonococcal Pathogenesis

Through the co-evolution of *N. gonorrhea* and humans, gonococcus has become highly adept at evading host immune defenses. These pathogenic mechanisms have important clinical correlates in terms of infectivity, disease manifestations, development of antibiotic resistance, and the potential for vaccine development.

A number of characteristics are known to contribute to the virulence of *N. gonorrhoeae* including pili, porin protein, opacity proteins, lipooligosaccharides, reduction modifiable proteins, IgA proteases, and iron- or oxygen-repressible proteins. A detailed discussion of these gene products and molecular systems are available elsewhere [4, 5]. While some gonococcal pathogenic mechanisms are well described, others remain unclear. For example, the recently discovered horizontal gene transfer between humans and *N. gonorrhea* is currently of unknown significance, beyond its affirmation of the extended length of time the two have co-existed [1].

Acquired through sexual contact, *N. gonorrhea* establishes infection of the urogenital tract by interacting with non-ciliated epithelial cells (Fig. 19.1). The molecular mechanisms responsible for gonococcal infection appear to differ between males and females. For example, invasion of female but not male urogenital cells is facilitated by cervix-specific CR3 complement receptors. Male urethral epithelial cells do not express these receptors. CR3 mediated invasion, in turn, is associated with asymptomatic cervicitis, perhaps explaining the higher incidence of asymptomatic disease in women than men [6]. The formation of biofilms on cervical cells may also play a role in the pathogenesis of *N. gonorrhea* in women [7].

After adhering to epithelial cell surfaces, gonococcus is engulfed into cells where it replicates intracellularly within phagocytes. The microbe later exits from the basal surface via exocytosis and enters the submucosal space. Mucosal epithelial damage and submucosal invasion lead to the influx of neutrophils with the resultant production of purulent material (e.g., "drip") into the lumen of infected tissues [4]. Both organism-specific factors, including peptidoglycan and lipopolysaccharide, alongside host-related responses, such as phagocyte production of tumor necrosis factor, contribute to tissue damage [5].

While the majority of invading gonococci are ingested and destroyed by neutrophils, a minority escape innate and early adaptive immune responses, thereby, allowing for persistent infection. Infrequently, disseminated infection occurs [8]. Normally, human serum is capable of killing
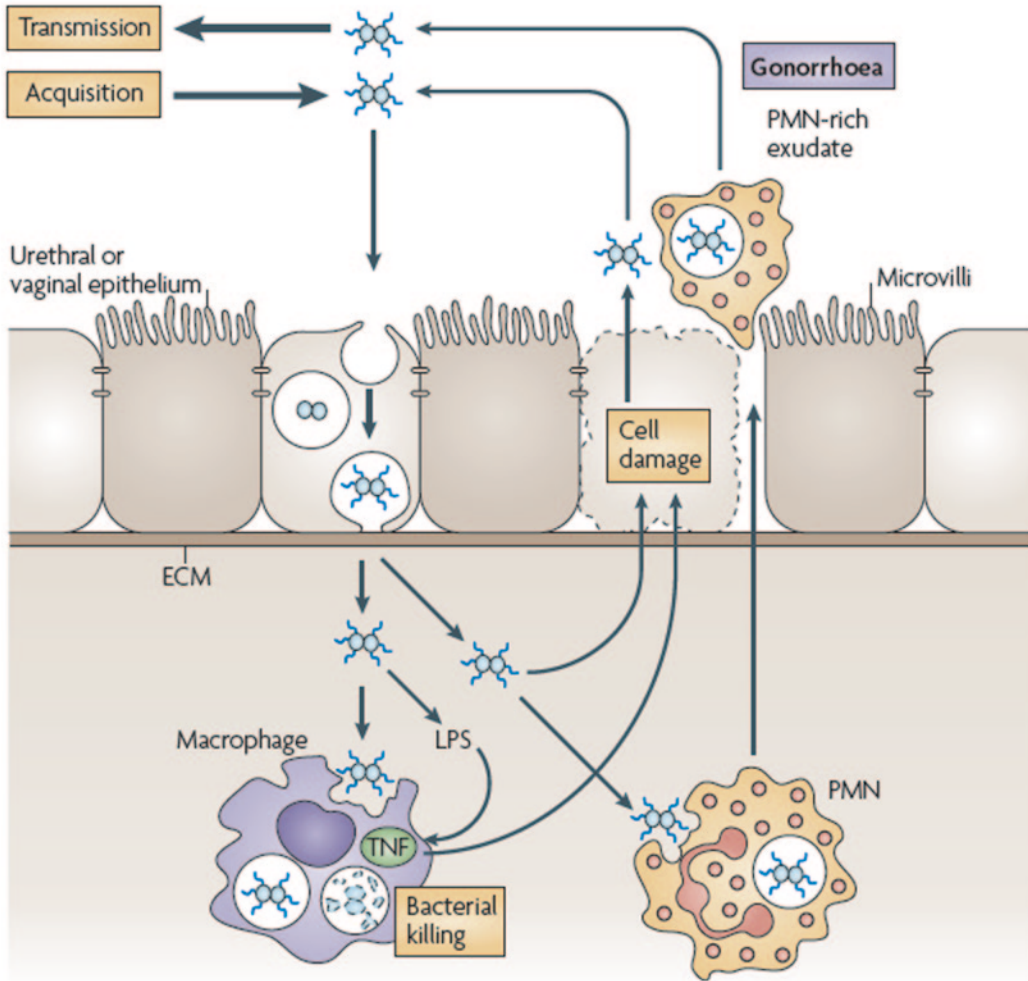
Fig. 19.1 Pathogenesis of gonococcal infection [5]. (With kind permission from Springer Science+Busness Media B.V. and M Virji)

circulating and mucosal gonococci via the activation of complement. However, some gonococcal strains are serum resistant, inducing defective terminal complement deposition. For example, gonococcus is able to bind C4BP, a key inhibitor of the classical pathway, thereby eluding complement-mediated lysis [9].

Recurrent gonococcal infection is the rule rather than exception. Although natural infection prompts antibody production against gonococcal cell wall components, the impact of the humoral immune response tends to be minimal. Antigenic and phase variation of outer gonococcal components, alongside mimicry of host glycolipids, fur-

ther limits an effective immune response [4, 5, 10] and is a major reason why vaccines developed using traditional techniques have failed. Additionally, interspecies and as well as interstrain genetic exchange within the *Neisseria* genus continually introduces novel antigenic components [4] including antibiotic resistance genes.

19.3 Epidemiology

Gonorrhea remains a significant public health concern worldwide. An estimated 88 million people are newly infected on a yearly basis with

Table 19.1 Global gonorrhea epidemiology: estimated new cases of adult gonorrhea infection (in millions) in 1995 and 1999. (Adapted from [27])

Region	1995 (in millions)	1999 (in millions)
North America	1.75	1.56
Western Europe	1.23	1.11
North Africa and Middle East	1.54	1.47
Eastern Europe and Central Asia	2.32	3.31
Sub Saharan Africa	15.67	17.03
South and South East Asia	29.11	27.2
East Asia and Pacific	3.27	3.27
Australia and New Zealand	0.13	0.12
Latin America and Caribbean	7.12	7.27
Total	62.15	62.35

Table 19.2 Global gonorrhea epidemiology incidence per 100,000 population in selected countries over time. (Adapted from [12, 14, 107, 140–142])

Country	1980	1995	1999	2008
Austria	92.62	11.29	5.44	9.85
Azerbaijan	32.64	26.2	12.08	13.38
Belarus	116.94	165.18	97.7	56.5 (2007)
Canada	223	19	14.9 (1997)	33.1 (2009)
Croatia	58.54	1.11	0.99	0.25
Czech Republic	86.46	19.73	10.28	7.63
Denmark	215.5	5.55	6.28	7.26
Estonia	237.36	200.22	83.09	10.88
Finland	200.5	7.4	4.94	3.79
France	–	0.35	0.57	1.7
Georgia	46.64	23.69	12.43	15.88
Germany	129.07	4.99	2.69	–
Israel	22.53	0.76	3.98	4
Italy	3.57	0.55	0.5	0.29
Norway	257.02	3.99	4.26	6.31
Poland	104.03	4.04	2.08	0.87 (2007)
Romania	91.1	24.71	17.76	2.95
Russian Federation	196.34	172.81	118.5	56.38
Switzerland	23.98	3.77	4.61	12
UK and Northern Ireland	98.73	17.95	28.62	31.33 (2007)
United States	442.1	147.5	129.2	111.6

the majority of cases occurring in the developing world [11]. The reported rate in the developing world is about 20 times the rate in industrialized countries (Table 19.1). Gonorrhea is the second most commonly reported bacterial sexually transmitted infection in many industrialized countries including the United States (US), Canada, and the United Kingdom (UK) [12–14]. While prevalence and incidence statistics are available for many countries, the current methods of data collection have limitations and likely substantially underestimate infection rates [15–17].

In general, surveillance data tends to be more robust from countries with national reporting systems such as those found in many industrialized countries including the US, Canada, the UK, Australia, and the Netherlands [18]. For example, the Gonococcal Isolate Surveillance Project (GISP) serves as a sentinel surveillance system in the US, monitoring gonococcal antimicrobial susceptibilities though ongoing testing of a select segment of the US population. Additionally, a number of European countries share a surveillance program called the European Gonococcal Antimicrobial Surveillance Programme (EURO-GASP), part of a broader European STI surveillance network known as the European Surveillance of Sexually Transmitted Infections (ESSTI). A number of these national and multinational systems collect detailed epidemiological and behavioural information in addition to antimicrobial resistance data [19], aiding in informing preventative

as well as treatment strategies [20]. Although an important step in the right direction, surveillance systems remain prone to inaccuracies and the paucity of data from developing countries hinders global evaluation of gonococcal rates and assessment of intervention programs [17, 21, 22]. Strengthening of local country data collecting systems and international collaboration are needed to improve surveillance of gonorrhea on a global scale. Table 19.2 compares rates of gonorrhea in selected countries in Europe with Canada and the United States. The variation in reported rates per 100,000 population is quite wide ranging from a low of 0.25 and 1.7 in Croatia and France, respectively, to much higher rates of 56.38, 56.5 and 111.6 in the Russian Federation, Belarus and the US, respectively.

Fig. 19.2 Example of an extensive social network among young people in Denver, US [38]. (With permission from A.A. Al-Tayyib)
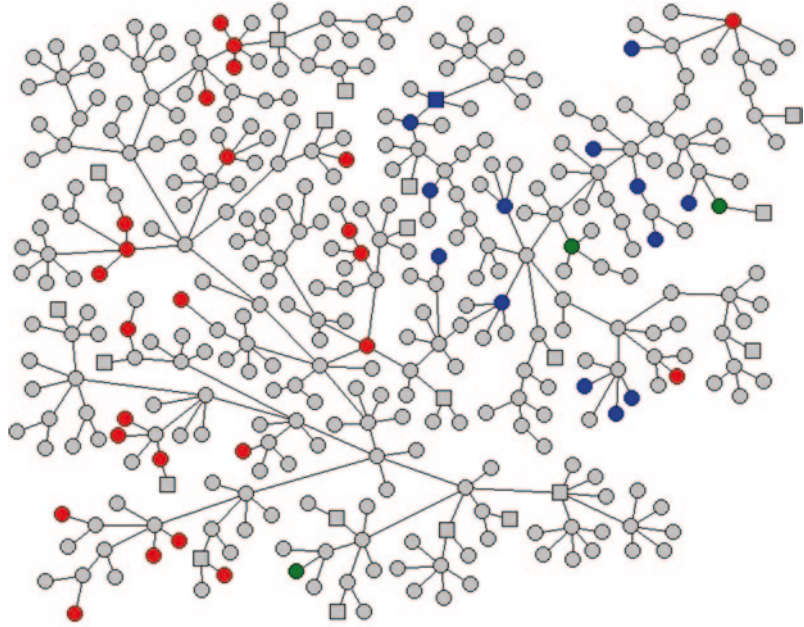
Comprehensive reviews of the historical epidemiology of gonorrhea are available elsewhere [17, 23]. In brief, gonorrhea rates have fluctuated worldwide over the last hundred years. Peaking around the Second World War in the 1940s and during the "sexual liberation" of the late 1960s and early 1970s, declining gonococcal rates were subsequently observed in the US, Canada and many European countries [12, 24, 25]. Following a transient rise in the 1990s and early 2000s, infection rates have since stabilized or declined in some countries but now show resurgence in others. For example, while gonorrhea rates have fallen in the US, they have risen in Canada [12, 14]. High infection rates also persist in many lower-income countries including Eastern Europe, the Western Pacific, Asia, Africa and Latin America [26–28].

19.4   Risk Groups: Emphasis on Young People & Sexual Networks

The factors needed to sustain a sexually transmitted infection epidemic are summarized by the case-reproduction ratio (Ro), an equation used to determine the number of new cases generated by an infected person. This ratio is calculated by the following equation: $Ro = \beta CD$, where "β" indicates the efficiency of transmission, "C" the number and/or rate of sexual partner change, and "D" the average duration of infectivity [29]. When Ro equals one, the infection is endemic and stable. Ro values greater than or less than one reflect increasing or decreasing infection rates, respectively.

Each component of the case-reproduction ratio reflects a variety of sexually transmitted infection risk factors. For example, the efficacy of transmission ("β") is determined, in part, by gonococcal strain subtype and infectious inoculum [30]. To illustrate the latter, male to female genital-to-genital transmission is more efficient than the reverse (e.g. male to female: 50–70 %; female to male: 20 %), likely due to a higher infectious inoculum in male urethral discharge than vaginal secretions [5, 31, 32]. Host behaviours, such as condom use, also play a role in transmission efficiency ("β") [33–35].

The variable duration of infectivity ("D") reflects the time between sexually transmitted infection acquisition and successful treatment or clearance of infection. Gonorrhea is mild or

asymptomatic in up to 50 % of infected females and in less than 10 % of infected males [36], delaying or preventing diagnosis, especially in women. Fear of stigmatization, inaccessible diagnostic and treatment services, and therapy non-compliance are other potential reasons for longer periods of infectivity and, hence, increased risk of transmission [17].

The last variable in the case-reproduction ratio, the number and/or rate of sexual partner change ("C"), is self-explanatory. However, this variable is worth exploring further from the standpoint of sexual networks. Long term monogamous sexual relationships are not part of a sexual network. Rather, sexual networks are characterized by core groups of people with frequent partner exchange and high degrees of connectivity [37]. These networks can be remarkably extensive as recently demonstrated by Al-Tayyib & Rietmeijer [38] (Fig. 19.2). Although some sexually transmitted infection cases are detected and treated, infection within the network remains making re-infection probable. Behavioural factors, such as sporadic condom use within the group, promote rapid spread and persistence of sexually transmitted infections [39]. More prevalent in core groups than the general population, gonorrhea is an archetype of sexually transmitted infection transmission and persistence within sexual networks.

Teenagers and young adults, due to a combination of social, behavioural and biological factors, are disproportionately affected by sexually transmitted infections [40]. Along with men who have sex with men, street youth, intravenous drug users, and persons in correctional facilities, young people often fit the "core group criteria" of sexual networks [23, 41]. Contributing teen behaviours include multiple concurrent sexual partners [39, 42–47], sequential sexual partnerships of limited duration [40, 48, 49], failure to use barrier protection and the mixing of alcohol, drugs and risky sexual activity [47]. Additionally, the smaller introitus and cervical immaturity of young females confers a biological risk of sexually transmitted infection acquisition [50].

The 2009 National Youth Risk Behavior Survey in the United States reported that 46 % of grade 9 (ages 14–15 years) to grade 12 (ages 17–18 years) students had engaged in sexual intercourse at least once, 34.2 % were currently sexually active (i.e., had sexual intercourse with at least one person during the 3 months prior to the study), and 13.8 % had had sex with ≥4 people. Of the 34.2 % of currently sexually active teens, 61.1 % reported condom use by themselves or their partner and 21.6 %, alcohol or drugs before the last sexual intercourse. Of the teens surveyed, 7.4 % reported being forced into sex [47].

Early sexual debut further puts teens at risk [51, 52] with 25 % of young people acquiring their first sexually transmitted infection within a year of first sexual intercourse [49, 53]. Although the 2009 US National Youth Risk Behavior Survey showed improvement in some measures as compared to a decade ago (e.g., 2000—49.9 % reported ever having had sexual intercourse; 16.2 %, having ≥4 lifetime sexual partners) [54], the sexual behaviours of young people continue to put them at increased risk for sexually transmitted infections including gonorrhea.

Gonorrhea rates also vary by racial or ethnic group and by geographic region most likely reflecting background differences in rates within sexual networks. For example, in the US and UK, black ethnic population groups have disproportionately higher gonococcal infection rates [12, 19] than whites. In the US, the gonorrhea rate is 20 times higher in blacks than whites with young black women bearing the heaviest gonorrhea burden [12]. Geographically, gonorrhea rates vary by population density and region. For example, gonorrhea is disproportionately concentrated in London in the UK, and in the South in the US [12, 13].

19.5 Clinical Presentation

The clinical presentation of gonorrhea is well-described elsewhere [55]. Rather than provide a comprehensive overview, only a brief description of infectious manifestations in teenagers and young adults is provided below. A discussion of gonorrhea in neonates and young children is available elsewhere [56].

19.5.1   Genitourinary Infection

As noted above, gonococcal infection in males is asymptomatic in 5–10 % of cases. Symptomatic males most commonly suffer from urethritis, which begins 2–7 days after exposure. Complications are rare in men. Gonorrhea in females, on the other hand, is mild or asymptomatic in up to 50 % of cases. Symptomatic females usually develop cervicitis, typically 3–5 days after exposure. Complications are more common in women than men with the most feared, pelvic inflammatory disease, occurring in 10–20 % of untreated females [57]. Additionally, gonococcal infection during pregnancy poses a risk to both the mother and unborn child [58, 59].

19.5.2   Pharyngeal & Anorectal Gonorrhea

Pharyngeal gonorrhea results from orogenital contact. Although historically considered to only affect men who have sex with men and commercial sex workers, the increasing rates of orogenital sex amongst heterosexual couples has escalated their risk [60]. Pharyngeal gonorrhea is usually clinically silent, tends to resolve spontaneously within 10–12 weeks, and rarely causes complications [59]. However, pharyngeal infection, if not treated, has been linked to disseminated gonorrhea and serves as a potential reservoir for transmission to sexual partners [60]. Additionally, through the genetic exchange between commensal and gonococcal *Neisseria* species, gonococcal infection of the pharynx facilitates antimicrobial resistance transfer [61].

In males, anorectal gonorrhea occurs almost exclusively from receptive rectal intercourse amongst men who have sex with men. In women, transmural inoculation from vaginal secretions is likely the most common source [62]. Like pharyngeal gonorrhea, anorectal infection is asymptomatic in the majority of cases but serves as an infectious reservoir for transmission to sexual partners [63], a site for microbial genomic exchange and for persistence within sexual networks.

19.5.3   Disseminated Gonococcal Infection (DGI)

Disseminated disease requires bloodstream invasion from local sites of gonococcal infection. However, signs and symptoms of mucous membrane involvement rarely precede disseminated infection. Disseminated gonococcal infection is a rare entity, estimated to occur in only 1–3 % of infected adult patients [59]. Although usually clinically silent, disseminated gonococcal infection can be symptomatic, typically causing mild to moderate illness. Even without treatment, symptoms usually resolve in a matter of days and long-term sequelae are atypical [64]. Infrequently, disseminated gonococcal infection can cause fulminant sepsis and death [65]. Disseminated disease is also associated with infection at other sites, most commonly septic arthritis. Less frequently, disseminated gonococcal infection presents as a triad of tenosynovitis, dermatitis and polyarthralgias without purulent arthritis [64]. Given an association between disseminated gonococcal infection and terminal complement deficiency, patients with recurrent disseminated disease should be tested for immune deficiency [66].

Other forms of disseminated gonococcal disease, including osteomyelitis, meningitis, endocarditis and myocarditis, are infrequently reported [59, 61].

19.6   Diagnosis

19.6.1   Screening

Screening decisions should be based on a solid understanding of epidemiology of gonorrhea. Given that gonorrhea rates vary considerably within and between populations, mass screening for this pathogen has largely been abandoned [23]. While some countries limit testing to symptomatic individuals and their sexual contacts, most recognize the need to extend gonorrhea testing to subpopulations with high prevalence rates and/or risk factors. For example, numerous organizations in the US support

Table 19.3 Comparison of Gonorrhea Diagnostic Tests

	Culture based	Molecular testing
Specimen transport	Requires rapid transport and stringent control of environmental conditions	Less stringent requirements. Testing able to detect nonviable organisms. More amenable to self-collected specimens. Allows batched, centralized testing
Minimum turn-around time	1–3 Days	Hours
Equipment	Agar plates, incubator, basic confirmatory tests	Commercial molecular detection platform +/− nucleic acid extraction system.
Enables antibiotic susceptibility testing	Yes	No
Cost per test	Inexpensive	Variable
Training	General clinical microbiology training	Molecular microbiology training
Simultaneous Chlamydia detection	No	Available on several platforms

routine testing of pregnant women with risk factors, men who have sex with men, and adolescent patients [67]. Less agreement has been reached over screening groups with low prevalence rates but high risk of asymptomatic, complicated infections (e.g., low risk pregnant women). Additionally, optimal screening intervals for at risk populations and the need for post-treatment testing requires further research.

Certainly, both the potential benefits and harms of screening must be considered prior to implementing a testing program. In favor of screening is the identification and subsequent treatment of asymptomatic patients, thereby, preventing disease complications and infectious spread to others (i.e., decreasing the prevalence in a sexual network). However, screening tools must be carefully selected, considering both the sensitivity and specificity of the test, alongside its applicability to the population under investigation [68]. The inverse relationship between disease prevalence and false positivity rate is a well recognized statistical phenomenon. In the context of sexually transmitted infection testing, false positive results are expected to cause needless patient anxiety, potential partner discord, and increase unnecessary antibiotic use. Hence, the limitations of a screening test must always be considered prior to its application.

Given the rise of multi-resistant gonorrhea (see below), the role of screening as a secondary prevention strategy may need to be reevaluated.

19.6.2 Microbiology/Diagnosis

Table 19.3 compares two current microbiological tests, culture and nucleic acid amplification tests (molecular testing), for gonorrhea, noting specimen transport requirements, potential for self collection, rapidity of test, necessary equipment, availability of antibiotic sensitivity testing, relative cost and expertise needed, and ease of coincident chlamydia detection.

19.6.2.1 Diagnosis of Genitourinary Gonorrhea

Gram Stain
Gram stain is a rapid tool for diagnosis of gonococcal urethritis in symptomatic men. *N. gonorrhoeae* characteristically appear as Gram-negative intracellular diplococci within polymorphonuclear cells. The coupling of opposing flattened bacterial surfaces produces the organism's distinctive "kidney bean" shape. In men with symptomatic urethritis, Gram stain has comparable specificity to culture [69]. While a presumptive diagnosis of gonorrhea in this demographic can

be made on the basis of Gram stain alone, a negative result does not rule out gonococcal infection [59]. Furthermore, nonurethral specimens from nonsterile sites are not appropriate for Gram stain due to poor specificity.

Culture

Culture remains an option for gonorrhea testing from genital sites. The appropriate site(s) for specimen collection depend on patient age, gender, and sexual practices as well as clinical manifestations of infection. While the primary site of culture collection from women has been the endocervix, alternative sample sites include the urethra and vagina. Vaginal or urine specimens are recommended for women post-hysterectomy and for prepubertal girls. The primary collection site from men is the urethra. Rectal and pharyngeal swabs should be collected in patients who have engaged in oral and anal intercourse, respectively. Conjunctival cultures should be sent in the cases of suspected gonorrhea ophthalmia. Lastly, blood, synovial, and cerebrospinal fluid specimens should be considered in suspected disseminated disease.

Culture is performed on selective media and incubated in carbon dioxide at 35–37 °C for up to 72 h. Colonies are generally heterogeneous in appearance reflecting variations in piliation and are both catalase and oxidase positive. A variety of options exist for confirmation including carbohydrate utilization, chromogenic enzyme substrate tests, immunologic methods and deoxyribonucleic acid (DNA) probe confirmation tests. Culture has several advantages including acceptable sensitivity, high specificity, relative low cost, and suitability for a variety of clinical specimens. It does not require the costly instrumentation associated with molecular detection and provides a viable organism for antibiotic susceptibility testing and tracking epidemiological trends. There are, however, multiple disadvantages to culture. It requires more invasive sampling techniques; in particular, urethral swabs for men and endocervical swabs for women. Culture also necessitates strict storage and transportation conditions given that *N. gonorrhoeae* has specialized growth requirements and is highly susceptible to

environmental variations. Lastly, culture takes 24–72 h to produce a definite result.

Nucleic Acid Amplification Diagnostic Techniques (NAATs)

The first non-culture diagnostic tests were based on the detection of specific gonococcal antigens. Suboptimal performance characteristics and high cost favoured the continued use of culture [69]. Antigen-based tests were subsequently supplanted by nucleic acid amplification tests (NAATs). Although more expensive than culture, nucleic acid detection methods offer a number of advantages over culture including rapid turnaround time and the ability to detect organisms that are nonviable for culture. NAATs are based on detection of species-specific DNA or ribonucleic acid (RNA) sequences. The enhanced sensitivity of NAATs permits testing on a wider variety of specimen types including urethral swabs, endocervical and vaginal swabs as well as urine. A variety of commercial NAATs, involving different amplification techniques and nucleic acid targets, have been approved for the detection of gonorrhea in most developed countries. While NAATs have been approved for endocervical, urethral, vaginal, and urine specimens, culture has been the standard for pharyngeal and anorectal specimens [70]. However, this situation is in transition as increasing data validates the use of NAATs for non-urogenital sites [71, 72].

NAATs sensitivity and specificity depends upon the amplification of organism-specific nucleic acid sequences. The success of a particular NAAT relates to the choice of target gene. Commercial and in-house NAATs have used a variety of genetic targets including the cryptic plasmid (cppB gene), opa genes, cytosine DNA methyltransferase and the 16SrRNA gene. With the potential exception of urine specimens, NAATs generally offer superior sensitivity to culture, attributable to their ability to produce a positive signal from as little as a single copy of target DNA or RNA [69].

NAATs have replaced culture in many countries as the preferred method of diagnosing gonorrhea from genital sites. Many assays offer the advantage of testing for both gonococcal and

chlamydia from a single swab or urine speciman—a benefit for both patients and clinicians. The ability to test less invasive specimens such as urine samples and vaginal swabs has a significant advantage over culture as it improves patient acceptance of testing [73] and provides the additional option of self-collected specimens—which many patients find preferable [74, 75].

Theoretical limitations to NAAT sensitivity are assay-specific. Interstrain genetic differences may result in false negatives due to variable or absent nucleic acid sequences. Given that gonococcal strain distribution varies geographically and temporally, the performance of NAATs may differ both among and within groups over time. However, in general, commercial assays have targeted very stable genes to minimize this issue. Competitive amplification, whereby targets of lesser concentration fail to amplify in multiplex assays, and the presence of inhibitors may also reduce assay sensitivity [70]. Positive internal controls identify some of these technical limitations. Notably, commercial NAATs do not routinely detect antibiotic resistance genes although these may be future targets.

NAATs typically exhibit high specificity. Interspecies homology within the *Neisseria* genus [76] and frequent horizontal genetic exchange among *Neisseria* species [70] are theoretical causes of false positive results. Cross-contamination during processing is a risk that must be controlled for with any NAAT to maintain test specificity. Maintaining high specificity is essential in order to maximize the positive predictive value of the test, particularly in low prevalence populations. New multiplex NAATs, which target multiple genes in a single assay, may provide improved target specificity. Additionally, limiting screening programs to high prevalence populations will maximize the positive predictive value of the utilized test.

19.6.2.2 Diagnosis of Pharyngeal and Rectal Gonorrhea

Cultured pharyngeal and rectal swabs generally have poor sensitivity [60]. Several studies have demonstrated the superior sensitivity of NAATs over culture in detecting pharyngeal and rectal gonococcal infection [71, 73, 77, 78]. In addi-

tion, although concerns have been raised regarding cross-reactivity with commensal *Neisseria* species at extragenital sites, many NAATs have been found to have superior specificity to culture. Ongoing validation of assays for the detection of pharyngeal and rectal gonorrhea will help to minimize potential reservoirs of infection and, secondarily, reduce the development of antimicrobial resistance (pharyngeal gonorrhea).

19.6.2.3 Diagnosis of Disseminated Gonococcal Infection

The yield from cultures is limited in disseminated gonococcal infections. However, it is prudent to culture all potentially infected areas, including genitourinary, rectal, and pharyngeal sites. The presence of a septic joint should prompt arthrocentesis. Mean synovial fluid leukocyte count in disseminated gonococcal infections is typically around 50,000 cells/mm^3 although it can be less than 10,000 cells/mm^3 [64]. Synovial fluid gram stain and culture are often negative. However, molecular testing of aspirated joint fluid with 16S rDNA PCR can augment diagnostic yield [79, 80].

Although blood cultures are negative in 50–75 % of cases, at least two blood specimens should be sent for culture in patients presenting with suspected disseminated disease [64]. The positivity rate may be higher in patients with tenosynovitis, dermatitis, and polyarthralgias than those presenting with purulent arthritis. Associated cutaneous lesions can be swabbed for culture but are also of low diagnostic value [81].

19.7 Gonococcal Resistance & Treatment

19.7.1 Resistance History

Since the introduction of antibiotics in 1937 [82], *N. gonorrhea* has evolved multiple resistance mechanisms, both chromosomal- and plasmid-mediated. The rise in gonococcal resistance can be attributed to both the adaptability of the organism (see pathogenesis above) as well as antibiotic prescribing habits. With regards to the latter, the increased use of over-the-counter and

broad spectrum antibiotics combined with inappropriate prescribing policies for many clinical situations have facilitated the emergence of gonococcal resistance.

Introduced in the 1930s, sulfanilamide was the first curative treatment for gonorrhea. However, antibiotic resistance developed rapidly [82] such that penicillins replaced sulfanilamides as first-line gonococcal therapy in the 1940s [83]. Although penicillin remained the mainstay of therapy for several decades, low-level resistance slowly developed with progressive increases in penicillin minimal inhibitory concentrations necessitating higher treatment doses [84]. Tetracycline, used initially to treat penicillin allergic patients with gonorrhea, also became less effective over time [85]. In the late 1970s, the emergence of highly-resistant, penicillinase-producing *N. gonorrhoeae* marked the end of penicillin as a therapeutic agent for gonorrhea [86].

Starting in the mid-1980s, quinolones replaced penicillins as the first-line treatment for gonorrhea [87]. Effective single oral dosing, a favorable side effect profile, and excellent efficacy at all anatomical sites made quinolones an attractive therapeutic option. However, isolates resistant to quinolones began to appear by the 1990s, initially in Asia and then quickly spreading—likely initially via travelers and then endemically within high risk groups—to Australia, Hawaii and later to North America and Europe [61, 88]. By the mid- to late-1990s, quinolones were no longer the drug of choice for gonorrhea in most Asia-Pacific countries and by the mid to late 2000s, England, Europe and North America followed suit [73, 89].

Of note, despite a decrease in the use of quinolones for treatment of gonorrhea in the UK, the rate of resistance has continued to climb in England [90] highlighting the resistance problem with gonorrhea. Because gonococcal infection is relatively common and the organism readily shares resistance genes, stopping particular antibiotic treatment of gonorrhea does not result in a diminution of resistance. The gonococcus continues to share resistance genes and still experiences ongoing resistance selection pressure because these antibiotics are widely used to treat other infections.

Extended-spectrum cephalosporins are currently considered first-line therapy for gonorrhea in most countries. Although cephalosporins remain largely effective, growing resistance has been noted throughout the Asia-Pacific, North America and Europe [21, 61, 91–98]. Both the UK and US based surveillance programmes have reported a minimum inhibitory concentration creep to both ceftriaxone and cefixime in recent years [21, 91, 99]. For example, in the US, from 2000 to 2010, the minimum inhibitory concentrations to cephalosporins increased from 0.2 to 1.5 % for cefixime and from 0.1 to 0.4 % for ceftriaxone in 2000 and 2011, respectively [91, 92]. Cases of treatment failure have been reported after oral cephalosporin use in Asia, the UK and Norway [94, 99–101]. Although suspected treatment failure with ceftriaxone is rarely reported [102, 103], a highly multi-resistant *N. gonorrhea* strain has recently been identified in a sex worker in Japan [104].

Considering the inability to prevent spread of resistant gonococci in the past, the emergence and spread of multi-resistant cephalosporin strains appears probable. Given global travel and sexual tourism this likely will not take long. With no effective single or combined antibiotic treatment to replace cephalosporins, gonorrhea may soon become untreatable. The obvious need for new antibiotic regimens, as the microbe becomes more resistant, is an area that has not received the research attention it deserves. Although the need has clearly been recognized for a number of years [105], novel antibiotic regimens for treating gonococcal infection remain inadequately addressed.

Potential strategies to combat cephalosporin resistance include the promotion of more appropriate antimicrobial use, the development of novel treatment agents and regimens, improved surveillance and reporting networks to facilitate the identification and rapid containment of resistant isolates, as well as more effective sexually transmitted infection control and prevention programs [2, 3, 21, 61, 106].

Table 19.4 Example of Gonorrhea Treatment Guidelines for Selected Infection Sites: Centers for Disease Control and Prevention 2010 [79, 92]

Clinical indication	Recommended regimens
Uncomplicated genitourinary or anorectal gonorrhea	Ceftriaxone 250 mg IM in single dose OR, IF NOT AN OPTION Cefixime 400 mg PO in a single dose[a] PLUS Azithromycin 1 gm PO in sgine dose OR, IF NOT AN OPTION Doxycycline 100 mg PO × 7 days
Uncomplicated pharyngeal gonorrhea	Ceftriaxone 250 mg IM in single dose[a]
Adult gonococcal conjunctivitis	Ceftriaxone 250 mg IM in single dose
Disseminated gonococcal infection	Ceftriaxone 1 g IV q. 24 h × 7 days[b]

[a] Testing and treatment for chlamydia is recommended. Treatment regimens for chlamydia, Azithromycin 1 gm orally in single dose OR Doxycycline 100 mg a day for 7 days, is part of dual therapy recommended for gonorrhea
[b] Consider changing to oral therapy (Cefixime 400 mg PO BID) 24–48 h after clinical improvement

19.7.2 Uncomplicated Genitourinary and Anorectal Gonorrhea Treatment Recommendations

Given the differences among national guidelines and the variability of resistance rates between countries, the details on recommended therapeutic regimens have been kept to a minimum. Additionally, the discussion is limited to therapy for adolescent and adult gonorrhea. Unless indicated otherwise, treatment recommendations provided here reflect the recently updated 2010 Centers for Disease Control and Prevention Sexually Transmitted Disease Guidelines (outlined in Table 19.4) [79, 92].

As of 2012, the CDC no longer recommends cefixime as a first-line agent for the treatment of uncomplicated genitourinary and anorectal gonococcal infections. This decision is based on declining cefixime susceptibility among urethral *N. gonorrhea* isolates from the Gonococcal Isolate Surveillance Program. Dual therapy with intramuscular (IM) ceftriaxone and either a single dose of oral azithromycin or 7 day course of oral doxycycline is now the recommended first-line treatment regimen for genitourinary and anorectal gonorrhea.

In addition to treating coincident *Chlamydia trachomatis* infection, ceftriaxone combined with azithromycin or doxycycline may serve to improve treatment efficacy and delay the development and spread of cephalosporin-resistant gonococcal isolates. Azithromycin is the preferred agent over doxycycline given the ease and compliance of single-dose treatment regimens and lower prevalence of gonococcal resistance to azithromycin than tetracycline [91].

Patients should avoid sexual activity until their antimicrobial therapy is completed and their symptoms have resolved. In the case of persistent gonococcal infection following first-line therapy, further treatment advice should be sought from an infectious diseases expert.

19.7.3 Treatment of Pharyngeal Gonorrhea

Although usually self-limited, pharyngeal gonorrhea serves as a potential reservoir for infectious transmission and antimicrobial resistance [60, 61]. Hence, pharyngeal gonococcal infection should be treated. More difficult to eradicate than urogenital and anorectal gonococcal infections, current therapy options are limited to a single dose of IM ceftriaxone. Alternative injectable and oral antibiotics, including cefixime, have unreliable efficacy in this treatment context and should be avoided. As with genitourinary and anorectal gonorrhea, co-treatment with azithromycin and/or doxycycline is recommended for pharyngeal gonorrhea.

19.7.4 Treatment of Disseminated Gonococcal Infection

Treatment of DGI requires parenteral therapy with ceftriaxone or an alternative third-generation cephalosporin (e.g., cefotaxime or ceftizoxime). Oral therapy can be considered in patients whose illness is sufficiently mild to permit close outpatient follow-up. However, a patient with

uncertain diagnosis, complicated disease, and/or difficulty tolerating oral medications should be hospitalized for the initiation of therapy. Transition to oral cefixime can be considered for susceptible organisms following 24–48 h of clinical improvement on intravenous therapy.

There have been no controlled clinical trials with respect to the duration of treatment for DGI. Antimicrobial therapy should likely be for a minimum of 7 days or continued at least until clinical signs of infection have resolved. Patients with purulent arthritis require joint drainage and 7–14 days of antibiotic therapy [64]. Treatment of meningitis or endocarditis consists of ceftriaxone for 10–14 days or a minimum of 4 weeks, respectively.

19.7.5 Special Treatment Circumstances & Alternative Agents

Ceftriaxone is also first-line treatment for gonococcal infections in pregnant women. Patients with human immunodeficiency virus (HIV) should receive the same treatment for gonorrhea as persons without HIV infection.

A non-beta lactam antibiotic may be needed in the case of severe penicillin or cephalosporin allergy [108]. Potential alternative options to cephalosporins include quinolones, macrolides, and spectinomycin. However, treatment in these cases should be performed in consultation with infectious diseases and, if possible, directed by isolate susceptibility testing.

Alternative safe and effective single-dose injectable cephalosporin regimens are available for urogenital and anorectal gonorrhea and include ceftizoxime, cefoxitin (in combination with probenecid), and cefotaxime. None of these injectable alternatives offers any advantage over ceftriaxone for urogenital infection. Oral substitutes include cefpodoxime and cefuroxime axetil. However, alternative oral and injectable cephalosporins may provide suboptimal efficacy for pharyngeal gonorrhea. Furthermore, caution is necessary when using oral cephalosporins to treat urogenital and anorectal gonorrhea due

to increasing rates of gonococcal resistance to cefixime.

Developed and marketed specifically for the treatment of gonorrhea, spectinomycin is an effective alternative for genitourinary but not pharyngeal infection. Resistance tends to be rare and sporadic. However, spectinomycin is an expensive, injectable drug that is not readily available in many countries including the US.

Uncomplicated gonococcal infection can be effectively treated with high-dose azithromycin (2 gm PO once). However, significant gastrointestinal side effects and rapid development of gonococcal resistance necessitate the use of high-dose azithromycin only under special circumstances [79, 91, 109]. As noted above, a single does of azithromycin (1 gm PO) is now recommended *in combination* with ceftriaxone (250 mg IM) to treat gonorrhea. However, due to growing microbe resistance, treatment failures have been reported even on this combined antibiotic regimen [110].

Quinolones can be considered as second-line treatment agents. However, given high global resistance rates to this antibiotic class, treatment with a quinolone should be delayed until susceptibility results are available. Furthermore, quinolones are contraindicated in pregnant women and persons younger than 18 years of age.

In some parts of the world, aminoglycosides are used to treat gonorrhea. For example, gentamicin is the national first-line treatment for gonococcal infection in Malawi. It has also been used to treat gonorrhea in Mongolia [111–113]. The precise mode of action of aminoglycosides against gonococcus remains unclear. Despite uncertain microbiological resistance breakpoints and limited clinical resistance data, treatment failures with aminoglycosides appear rare [114, 115].

19.7.6 Treatment: Other Recommendations

Reassessment following treatment bestows a number of benefits (e.g., to confirm compliance, ensure resolution of symptoms, enquire about adverse reactions, provide further education and

partner notification). However, follow-up visits may not be practical in all settings [36].

As per the 2010 CDC guidelines and 2012 update, repeat testing is not recommended upon completion of gonorrhea treatment with first-line therapy [91, 92]. However, a test of cure should be considered in the following circumstances: if second-line agents are utilized, following treatment of pharyngeal gonorrhea, and/or if symptoms or signs of infection persist. Some countries, such as the UK, recommend a test of cure in all cases, recognizing the emerging problem of resistance to extended-spectrum cephalosporins [36].

Evidence pertaining to the method and timing of test of cure is limited. In cases of persistent symptoms or signs, a repeat culture should be performed at least 72 h after completion of antimicrobial therapy [116]. NAATs are expected to remain positive for weeks following successful treatment and do not allow for the identification of resistant organisms. Thus, a positive NAAT should be followed by a confirmatory culture whenever possible.

Given high rates of recurrence, the identification of *N. gonorrhea* following treatment is more likely to reflect reinfection rather than treatment failure [117–120]. With this in mind, retesting for re-infection 3 months after treatment is recommended, especially in high risk patients. Additionally, in the case of disease recurrence, further patient education and partner treatment should be pursued.

Chlamydia trachomatis co-infection is common in patients with gonorrhea. With this in mind, patients treated for *N. gonorrhea* should also be tested and treated for chlamydia. Other sexually transmitted infections should also be considered, including syphilis, HIV, and hepatitis B. The therapy of choice for chlamydia, azithromycin (1 gm PO once) or doxycycline (100 mg BID×7 days), corresponds to the dual therapy now suggested by the CDC for gonorrhea.

Positive gonorrhea cases should be reported to the local public health agency, as per national guidelines, and contact tracing pursued. Contact tracing reduces the risk of re-infection and facilitates the identification and treatment of asymptomatic infections [121]. All sexual partners exposed to an index case within 60 days of symptom onset or diagnosis should be evaluated and treated. If greater than 60 days has passed since the index case has had sexual contact, the most recent sex partner should be contacted. Contact tracing may be performed by patients, physicians or public health departments depending on local and national strategies. To achieve compliance with notification, creative maneuvers have been used. The sexually transmitted infection notification program in British Columbia, Canada (www.gender-focus.com/2011/08/08/std-notification-by-e-card-starts-in) uses humour to garner attention and interest. Patient-delivered therapy to sexual partners has been shown to decrease the rate of gonococcal reinfection [122]. However, this approach may be poorly received by some patients and/or partners. Patient-delivered therapy also leads to missed opportunities to educate sexual partners and to diagnose coexisting sexually transmitted infections. This is particularly problematic for men who have sex with men where the risk of coexisting sexually transmitted infections is high. Given ethical and medicolegal issues around partner delivered therapy, local laws should be reviewed before utilizing partner delivered therapy [123].

Even with well funded and supported public health and/or patient contact tracing, all infected contacts in a sexual network are unlikely to be tracked down as no one member of the network knows all members. Leaving an infected sexual network member untreated keeps the organism ricocheting around the network.

19.8 Prevention

Most, if not all, countries recognize gonorrhea as a public-health priority. However, the strategies employed to address this sexually transmitted infection vary widely. While early diagnosis and treatment is uniformly pursued, emphasis on prevention has varied among countries [23].

The prevention of sexually transmitted infections in young people has been reviewed elsewhere [124]. To summarize, few trials on the efficacy of sexually transmitted infection pre-

vention programs have been published within the last decade. Amongst published studies, considerable variability in program type and efficacy has been identified. While some prevention strategies have been associated with diminished infection-related risk behaviors, few report corresponding reductions in confirmed sexually transmitted infections. Given that rates of gonorrhea appear to be rising in some industrialized countries as noted earlier, this is especially concerning.

One well-studied preventative measure is condom use. Condoms, as well as other barrier contraceptive methods, provide a high degree of protection against sexually transmitted infections [33–35]. Post-exposure antibiotic prophylaxis also reduces risk of infection but is unlikely to be cost effective [125]. Another strategy, gonococcal mass treatment programs for at risk groups, has been trialed with limited success. Both antibiotic prophylaxis and mass treatment strategies carry the risk of fostering antimicrobial resistance, a serious concern given the emergence of multi-resistant gonococci [126, 127].

New and innovative approaches to prevention are required. In general, recent decades have seen a movement towards multidisciplinary and multileveled approaches with emphasis placed on the integration of gonorrhea prevention programs into already existing community and health-care structures [128]. Additionally, the empowerment of individuals and communities to develop and foster preventative strategies relevant to their particular social contexts is essential. Social networks influence the opinions and decisions of members [129]. Therefore, targeted core group counseling may provide the opportunity for better information dissemination within groups. For example, peer-led rather than outsider-initiated sexual health interventions (i.e., on condom use, contact tracing) are more likely to succeed, as demonstrated in adolescent and other populations [38, 130, 131].

Another important prevention strategy is the use of appropriate communication tools. Young people increasingly rely on the internet and social media as sources of health information [132–134]. With this in mind, sexual health edu-

cators need to develop creative ways of getting their message across to teens and young adults. For example, novel Internet-based strategies are being trialed in Alberta, Canada, to tackle the ongoing syphilis epidemic within that province. One approach utilizes a spoof on online dating (http://www.plentyofsyph.com) that has been associated with a significant increase in young people coming forward for testing [2]. Similar strategies could prove useful for other sexually transmitted infections including gonorrhea.

Lastly, more adolescent-friendly health care services are required. Teens tend to live in the "now" and are less likely to seek treatment at appointment-only clinics, especially if wait times are long [135]. Online triage systems and options for testing-only express visits may enhance the efficiency of busy sexually transmitted infection clinics [136, 137]. Drop in clinics, especially if located where young people frequent (e.g., malls and schools) are another option, allowing teens to follow through with "spur of the moment" decisions to get sexually transmitted infection testing.

Learning about sex, sexuality and the prevention of sexually transmitted infections is a basic human right of adolescents [138]. The strategy of "abstinence" or "abstinence until marriage" lacks scientific evidence and is flawed from a medical-ethical standpoint [139]. Given that sexually transmitted infections, including gonorrhea, disproportionately affect young adults, we have a duty to provide adolescents with meaningful knowledge and the skills to protect themselves from acquiring and transmitting sexually transmitted infections. This will require collaboration on many levels, ranging from the family unit and the community to local and national governmental levels.

19.9 Conclusion

Moving into the second decade of the 21st century, *N. gonorrhoeae* continues to represent a significant public health challenge. Gonococcal rates persist above national and international targets, resulting in needless morbidity and

mortality. The emergence of multi-resistant gonorrhea is only going to make this situation worse.

Although the development of NAATs has improved our ability to diagnose gonorrhea, at least in industrialized countries, further refinement of diagnostic techniques is required. In particular, the role of pharyngeal gonorrhea as a significant reservoir of infection and antimicrobial resistance necessitates the development of more effective easy to use diagnostic tools. Furthermore, because resistance is such an important issue, traditional culture and sensitivity testing remains essential for tracking evolving gonococcal resistance both nationally and globally. Sentinel sites where cultures are done routinely will be key.

N. gonorrhea has proved to be a highly adaptable organism. Increasing gonococcal resistance to cephalosporins, with no single dose alternative antibiotics in the pipeline, threatens to make this organism untreatable. Effective and prudent antimicrobial strategies alongside robust national and international gonococcal surveillance programs are very much in need.

Lastly, sexually transmitted infection prevention strategies have never been more important. Public health services should aim to address the behavioral and social factors that put people at risk, including youth-friendly education and health care services. As these move forward, rigorous evaluation and collaboration of prevention strategies will be required at local, national and global levels.

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Management of Severe Malaria: Results from Recent Trials

20

Peter Olupot-Olupot and Kathryn Maitland

Abstract

Globally, malaria remains a substantial public health burden with an estimated 349–552 million clinical cases of *P. falciparum* malaria each year—leading to 780,000 deaths directly attributable to the disease. Whilst the outcome from severe malaria in Africa children remains poor, recent developments in the management of malaria have come from two key sources—the introduction of new, safe and rapidly-effective anti-malarials and high quality evidence from two of the largest clinical trials ever conducted in African children with severe malaria. As a result, the time-honoured anti-malarial treatment for severe malaria, quinine, will now be replaced by artesunate, a water-soluble artemisinin derivative. Supportive care, specifically the management of shock, has been informed by a large late phase clinical trial which concluded that bolus resuscitation is harmful and therefore should be avoided in children with severe malaria, including the high risk group with severe metabolic acidosis and advanced shock.

20.1 Background

Of the five species of malaria that infect humans, *Plasmodium falciparum* causes most severe and fatal disease in the tropics. In recent years, *Plasmodium vivax* has been also been described as causing severe and complicated malaria in Asia [1].

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Globally, the most reliable recent estimate indicates that there were 349–552 million clinical cases of *P. falciparum* malaria in 2007 [2], with up to 780,000 deaths directly attributable to the disease [3]. The burden and spectrum of malaria varies from one geographical region to another, with intensity of transmission and between adults and children. There have been some encouraging reports of declining malaria incidence, but these relate to selected areas and may not reflect the general situation [4]. Better malaria control, early diagnosis and improved drug treatment may each contribute to changing malaria epidemiology but overall trends are not yet comprehensively understood. In areas with high malaria transmission,

Table 20.1 Clinical signs of severe malaria

High priority: emergency management		
Clinical	Laboratory	Intermediate risk: need for high dependency care
Hypoxia (oxygen saturations <92 %)	Severe anaemia haemoglobin <6 g/dl	Haemoglobin 6–10 g/dl
Respiratory distress	Hypoglycaemia <3 mmol	History of convulsions during this illness
Hypotension (systolic blood pressure <50, <60 or <70 mmHg if <1 year, 1–5 years or >5 years)	Metabolic acidosis (base deficit >8)	Hyperparasitaemia >5 %
Depressed conscious state (V, P or U on AVPU scale)	Lactate ≥5 mmol/l	Visible jaundice
Abnormal posturing	Creatinine >80 mmol/l	*P. falciparum* in a child with sickle cell disease
Inter-current seizures	Severe hyperkalaemia (potassium >5.5 mmol/l)	

incidence has not changed and in some areas increases in disease burden have been reported [5, 6].

Prompt diagnosis and treatment is crucial to prevent mortality especially in high-risk groups including young children and some visitors, who have little or no immunity. Travellers, of any age, from non-endemic areas are particularly at risk, including those formerly from endemic areas and returning for short or long visits to their home or other endemic areas, because naturally-acquired immunity wanes rapidly. In the past decade there have been substantial therapeutic advances in the management of both mild and severe disease, with newer drugs, in particular artemisinin based combinations replacing time-honoured chloroquine and quinine. These developments are welcome as parasite resistance to malaria treatments continues to threaten malaria control and effective case management globally. This chapter focuses on the management of severe malaria with specific reference to the latest developments in treatment.

In 2010 and 2011, two of the largest clinical trials ever conducted in African children with severe malaria were published, both with groundbreaking results [7, 8]. Most of the latter part of this chapter is dedicated to discussing these trials. Both manuscripts are openly accessible on-line and we recommend that readers refer to these manuscripts as necessary. By way of introduction and to put into to context the relevance of

the trials to the clinical management of paediatric malaria in Africa and, by extrapolation, to the management of children in the UK, we first describe the clinical spectrum of severe malaria.

20.2 What Defines Severe Malaria?

For decades severe malaria in African children had been rationalised into two non-over-lapping major syndromic presentations, those of cerebral malaria and severe malarial anaemia [9, 10]. Research over the years has established that severe acidosis, clinically manifesting as respiratory distress (Kausmaul's breathing), is a common complication and the leading independent factor predicting fatal outcome [11, 12]. Severe falciparum malaria has more recently been described as a complex syndrome, affecting many organ systems, which has many features in common with sepsis syndrome [13]. Indeed, clinical distinction between severe malaria and other common causes of life-threatening febrile illnesses including bacterial sepsis, pneumonia and CNS infection is often impossible at the time of presentation [14] (see Table 20.1 for summary of clinical characteristics). The most comprehensive description of the clinical spectrum of severe malaria in African children is the AQUAMAT trial [7]. The trial involved 11 centres in 9 countries including a mixture of urban and rural hospitals and populations with differing malaria endemici-

ties. All 5,425 participants in the trial had clinical evidence of severe malaria and the diagnosis was reinforced by quality controlled laboratory evidence of *Plasmodium falciparum* infection. Overall, at baseline, 37 % had coma, 32 % had convulsions, 9 % had compensated shock (capillary refilling time greater or equal to 3 s) and 3 % decompensated shock (systolic blood pressure < 70 mmHg and cool peripheries). Only 2 % had clinical jaundice. Among admission laboratory parameters, 30 % had severe anaemia (haemoglobin < 5 g/dl), 10 % were hypoglycaemic (blood sugar level < 3 mmol/l) and 43 % had severe acidosis (base deficit > 8). Co-morbidities included 13 % with (clinician diagnosed) sepsis (including 10 % with culture proven sepsis); 13 % with radiological evidence of pneumonia and 2 % with HIV, malnutrition or meningitis. Whilst dark urine (or haemoglobinuria also known as 'blackwater fever') and clinical jaundice were infrequent in these centres, there are other areas in Africa where these complications are more common (Olupot-Olupot, unpublished data). Key independent predictors of fatal outcome included acidosis, cerebral involvement, renal impairment and comorbidity [15].

We have recently published a review of the features of severe malaria and proposed UK management guidelines [16], which are based on information obtained from personal archives of references, the current World Health Organization (WHO) guidelines [17] and the Advanced Paediatric Life Support (APLS) guidelines [18]. Our recommendations for diagnosis are largely unchanged with the exception of shock—which we suggest should be defined by low systolic blood pressure, the rationale for which is discussed below as are the implications of recent results for therapeutic and supportive management recommendations.

20.2.1 Bacterial Infection

Comorbidity in children presenting to hospital is common in malaria-endemic Africa. Invasive bacterial disease is reported in 5–12 % of children with recent or current malaria, commonly Gram-

negative (GN) infections [19, 20]. However, the sensitivity of blood cultures to detect septicaemia is low, so it is likely that the figures are underestimates [21]. Non-typhoidal salmonellae (NTS) are the most common isolate, particularly in younger children suffering from moderate or severe anaemia [22, 23]. The mechanisms underlying this apparent susceptibility remain uncertain. Since children in malaria-endemic areas often present to hospital with malaria parasitaemia—there is a possibility that the association with bacteraemia is largely observer bias. However, the most persuasive data that there is a significant biological association come from the study of Nadjm and colleagues in Tanzania [20], describing the spectrum of bacteraemia in unselected children admitted to hospital. Three subgroups were described, children who were malaria-negative, cases with recent malaria (malaria antigen positive but slide negative) and children with positive malaria slides. Among malaria-negative children with bacteraemia, 51 % had Gram-positive organisms isolated and 49 % Gram-negative (GN). Among children with recent or intercurrent malaria 76 % of bacteraemias were with non-*Haemophilus influenzae* GN organisms (of which 90 % were NTS). There was also a trend towards increasing proportion of non-*Haemophilus influenzae* (and non-NTS) Gram-negative enteric ('other' GN) organisms with increasing density of malaria parasitaemia. However, invasive bacterial disease is much less commonly reported in imported malaria in non-endemic settings [20].

20.3 Recent Changes in the Management of Severe Malaria

20.3.1 The AQUAMAT Trial

20.3.1.1 Background

Quinine has been used to treat malaria for over three centuries and until recently was the mainstay of treatment in African children with severe disease. In South East Asia the problem of quinine resistance, the substantial case fatality of severe malaria in adults and the availability of artemisinin derivatives prompted the performance of a

large multicentre randomised trial (SEAQUA-MAT) comparing parenteral artesunate with quinine in four countries [24]. The trial included 1,461 patients, most of whom were adults, and was stopped early by the data monitoring and safety committee following a planned interim analysis, which showed a substantial benefit in favour of artesunate. Mortality in patients receiving artesunate was 17 % (107/730) compared to 22 % (164/731) in patients receiving quinine. This equates to a relative reduction in mortality following artesunate compared to quinine of 34.7 % (95 % CI 18.5–47.6 %; $p = 0.0002$). Since this trial, artesunate has been the drug recommended for treatment of severe malaria in adults by the WHO.

A separate trial in African children was justified on several grounds. First, there were important differences between the clinical manifestations, the tempo of the disease and the response to treatment in African children with severe malaria compared with adults in South East Asia. In severe malaria in children most deaths occur within the first 24 h—far sooner than in most adults in the SEAQUAMAT trial. This provides a much narrower time-window for any advantage of artesunate in killing young ring stage parasites more rapidly to translate into clinical benefit. Second, malaria parasites in Africa are more quinine sensitive, with no reported quinine resistance at the time of the trial. Third, drug cost and availability were also potential issues. Finally, previous trials in children in Africa comparing artemether with quinine found little evidence of superiority. However, artemether is an oil-based formulation of artemesinin, which needs to be given by intramuscular injection. Pharmacokinetic studies showed that the drug is only slowly and erratically released from the intramuscular injection site [25, 26] suggesting a possible reason for the previous failure to show a significant difference between quinine and artemisinin derivatives in African children. The advantages of intravenous (IV) artesunate include its potency compared to artemether [27] and instant bioavailability by both IV and intramuscular routes leading to rapid peak concentrations within 1 h [26, 28]. All these considerations

supported the need for a conclusive trial to establish best practice.

20.3.1.2 Study Design

The AQUAMAT was conducted in nine African countries, and ran between October 2005 and July 2010 when the recruitment target was reached. Participating hospitals included university hospitals, research institutes and rural hospitals without prior research experience. None of the facilities had intensive care units—so the large majority of children were managed pragmatically on general paediatric wards. Children were eligible if they were under 15 years old with clinical evidence of severe malaria and a positive rapid malaria test (Optimal) for *Plasmodium falciparum* malaria (indicating current malaria infection). Treatment, at recommended dosages, with parenteral quinine or an artemisinin derivative for more than 24 h before admission were the only exclusion criteria. The trial was an open label trial of both antimalarials given parenterally (either by intramuscular or intravenous administration). Randomisation was masked as study numbers were kept inside opaque sealed paper envelopes and opened in numerical order. The card inside directed the clinician to a separate, sealed trial pack which contained the assigned intervention was on the first page. Blinding was not possible owing to the different methods of drug dilution and administration—artesunate requiring a two-step procedure for preparation and intravenous quinine needs to be given as an infusion, whereas artesunate is given as an injection. The interventions were given until the child was able to take an oral combination treatment, Coartem® (Artemether-Lumafantrine)—which was used to complete the treatment course. The primary outcome was in-hospital mortality and secondary outcomes included the incidence of severe complications and neurological sequelae.

20.3.1.3 Results

By intention to treat 572 (9.7 %) of the 5,425 children enrolled in the trial died. Whilst baseline characteristics of the children in the two arms were similar, mortality was substantially lower in the artesunate group 230/2,712 (8.5 %) compared

to 297/2,713 (10.9 %) in the quinine arm. Comparing quinine to artesunate-treated children, the relative risk of death was 22.5 % (95 % CI 8.1–36.9) greater in the quinine arm ($p = 0.002$). There was no difference in outcome whether the drugs were administered intravenously or intramuscularly, nor were there any differences in outcome across any of the major clinical spectra defining severe malaria.

The development of coma and/or convulsion was more common during hospital admission in the quinine arm ($p = 0.02$); however there were no other differences between the arms in number of complications, additional treatments prescribed, time to recovery or numbers with neurological sequelae—which included 61 persistent mild, moderate or severe such sequelae among survivors.

20.3.1.4 Outcome: Change of Policy

Based upon the results of the SEAQUAMAT trial and now the AQUAMAT trial, parenteral artesunate is now recommended by the WHO and being adopted by individual countries as policy as the first line treatment for severe malaria in both adults and children. Artesunate should be given at admission then at 12 and 24 h, and once a day thereafter until the children can take and retain oral medication. Treatment must then be completed with a full course of artemisinin based combination therapy (ACT). If parenteral artesunate is not available, artemether or quinine are acceptable alternatives.

20.3.2 FEAST Trial

20.3.2.1 Background

The FEAST trial (Fluid Expansion As Supportive Treatment) was the first large controlled trial of fluid resuscitation comparing the use of early bolus resuscitation with local standard of care (no bolus). At the time this trial was conducted, the recommendations for fluid resuscitation in Africa were substantially different from those in other parts of the world [29] and use of fluid resuscitation was highly controversial in children with severe malaria [30–32]. Although there was

evidence to suggest that hypovolaemia contributes to the pathophysiology of severe malaria [33, 34], there were conflicting recommendations as to how it should be treated. Published WHO guidelines suggested that children might respond favourably to extra fluid in the presence of concomitant dehydration. However, specific recommendations regarding the type, rate or volume of fluid administration were not provided [35]. Use of invasive central venous pressure monitoring was recommended to monitor fluid replacement [35]. However, for the vast majority of children in sub-Saharan African hospitals this was neither available nor feasible. Therefore most such children with severe malaria were receiving no specific fluid management apart from blood transfusion in some cases, as this is recommended for the management of severe anaemia [36].

FEAST was designed to address this controversy [8]. However, severe malaria and bacteraemic sepsis syndrome are similar and simple bedside and laboratory assessment cannot distinguish between them [14, 37–40]. The FEAST trial was designed as a pragmatic trial incorporating both populations in order to provide practical information for hospitals with few diagnostic facilities.

20.3.2.2 Choice of Study Fluids

The trial was an open label randomised controlled trial of fluid resuscitation. Comparisons were at three levels; firstly, 0.9 % saline (a crystalloid) was compared with 5 % human albumin solution (colloid). Secondly, each of the fluids was compared to no bolus (control arm) and thirdly, both fluids were compared against no bolus (control). The inclusion of albumin (HAS) was based upon the results of three Phase II trials in Kenya comparing different fluids in children with severe malaria and signs of hypovolaemia [41–43]. The first trial had shown that up to 40 ml/kg of 0.9 % saline or HAS was safe and corrected haemodynamic indices of hypovolaemia [41]. The second trial was an open label, randomised comparison of saline and HAS in 117 children with severe malaria and acidosis. Mortality in children treated with HAS was 3.6 % (2/56), compared to 18 % (11/61) in those treated with
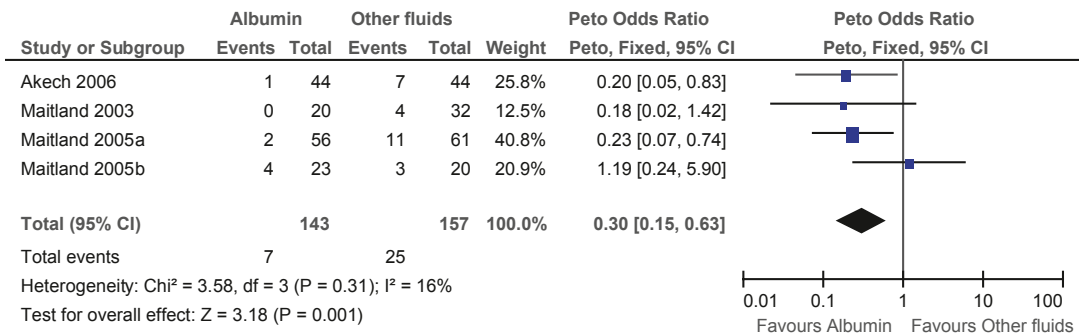
Study or Subgroup	Albumin		Other fluids			Peto Odds Ratio	Peto Odds Ratio
	Events	Total	Events	Total	Weight	Peto, Fixed, 95% CI	Peto, Fixed, 95% CI
Akech 2006	1	44	7	44	25.8%	0.20 [0.05, 0.83]	
Maitland 2003	0	20	4	32	12.5%	0.18 [0.02, 1.42]	
Maitland 2005a	2	56	11	61	40.8%	0.23 [0.07, 0.74]	
Maitland 2005b	4	23	3	20	20.9%	1.19 [0.24, 5.90]	
Total (95% CI)		143		157	100.0%	0.30 [0.15, 0.63]	
Total events	7		25				
Heterogeneity: Chi² = 3.58, df = 3 (P = 0.31); I² = 16%							
Test for overall effect: Z = 3.18 (P = 0.001)							

0.01 0.1 1 10 100
Favours Albumin   Favours Other fluids

Fig. 20.1 Forrest plots comparing effect of albumin versus other resuscitation fluids

saline ($p = 0.01$) [42]. In the subgroup of children with coma, only 1/21 (5 %) of children receiving HAS died, compared with 11/24 (46 %) who received saline ($p = 0.002$). Possibly owing to the greater survival rate in the HAS arm there was a non-significantly higher rate of neurological deficit (11 %; 6/54) compared to the saline arm (4 %, 2/50) [42]. In a further trial, mortality among children treated with HAS was lower (2.3 %; 1/44) than among those treated with the synthetic colloid Gelofusine (16 %; 7/44) [43]. In the sub group with coma, 1/ 25 (4 %) of HAS recipients died compared to 6/23 (26 %) in the Gelofusine arm ($p = 0.04$). Neurological sequelae complicated 3/43 (7 %) survivors receiving HAS and 1/37 (3 %) survivors receiving Gelofusine [43]. A meta-analysis of the combined data from the four trials (Fig. 20.1) demonstrated a reduced mortality with HAS compared to saline or other colloids (Peto odds ratio 0.30; 95 % confidence interval 0.15, 0.63; $p = 0.001$ and test for heterogeneity-$\chi^2 = 3.58$, $p = 0.31$) but also noted the potential with such small sample sizes to overestimate effect. On the basis of the evidence from these previous trials, it was decided to include randomisation to HAS in the FEAST trial.

20.3.2.3 FEAST Trial Design

Children aged >60 days presenting with severe febrile illness and signs of impaired perfusion in six centres in three East African countries (Uganda, Kenya and Tanzania) were eligible for inclusion. Cases of gastroenteritis, severe malnutrition and non-medical causes (burns and trauma) were excluded from the trial, since the design and interventions were not relevant to these easily identifiable clinical presentations. Children were enrolled in two strata according to systolic blood pressure at presentation: 3,141 children without severe hypotension were enrolled in stratum A and received either an immediate bolus of 20 ml/kg given over 1 h (later increased to 40 ml/kg after protocol amendment) of 5 % HAS (1,050 children) or 0.9 % saline solution (1,047 children) or no bolus as controls (1,044 children); stratum B enrolled 29 children with severe hypotension (systolic blood pressure <50 mmHg if <12 m; <60 mmHg if 1–5years; <70 mmHg if >5 years) were randomized to receive either 40 ml/kg (later increased to 60 ml/kg after protocol amendment) HAS or saline boluses—there were no control arm in stratum B. After 1 h saline-bolus and albumin-bolus arms, but not the control arm, received an additional 20 ml/kg bolus if signs of impaired perfusion persisted. Beyond this time point further fluid boluses in all three arms were only prescribed for severe hypotension (see above definition)—where an additional 40 ml/kg boluses of study fluid (saline for the control arm) was given over 1 h. No crossover between the saline and albumin was permitted [8].

20.3.2.4 Supportive Care

All children were managed on general paediatric wards. Ventilation facilities other than short-term 'bag and mask' support were unavailable. Training in triage and emergency paediatric life support was given throughout the trial to optimize case recognition, supportive management and ensure protocol adherence. Basic infrastructural support for emergency care, oxygen saturation

and automatic blood pressure monitoring was provided. All children received maintenance fluids, anti-malarials and/or antibiotics as required. Throughout the hospital admission time, episodes of hypotensive shock within 48 h and Adverse Events (AE) potentially related to fluid resuscitation Pulmonary Edema (PE), Raised Intracranial Pressure (RICP) and severe allergic reaction were actively solicited. AEs were sent to the Clinical Trials Facility, Kilifi, Kenya within 2 days where they were reviewed by an independent clinician. All AEs were subsequently monitored on site against source documents by visiting monitors. Twenty-eight-days reviews for neurological deficit were performed by an independent clinician, blind to treatment allocation. Children with neurological impairment at day 28 were re-assessed at 6 months.

The primary endpoint was mortality at 48-h after randomisation. Secondary endpoints included mortality at 28-days, neurological sequelae at 28-days and 24 weeks, episodes of hypotensive shock within 48 h of randomisation and adverse events related to fluid resuscitation (pulmonary oedema, intracranial hypertension or severe allergic reaction among those receiving HAS).

20.3.2.5   Results

Among the 3,141 children in stratum A, there were no major differences in baseline characteristics across the three arms. Median age was 24 months (IQR 13–38); 62 % had prostration, 15 % were comatose and 83 % had increased work of breathing. Moderate-severe acidosis was present in 1,070 (52 %) and severe lactic acidosis in 1,159 (39 %). Severely anaemic children were 987 (32 %) and 187 (6 %) had hypoglycaemia. Malaria was confirmed in 1,793 (57 %); 126/1,070 (12 %) had bacteraemia and 4.4 % were HIV seropositive.

In stratum A, 48-h mortality was 111/1,050 (10.6 %), 110/1,047 (10.5 %) and 76/1,044 (7.3 %) in HAS-bolus, saline-bolus and control arms, respectively. Risk ratios (95 % confidence intervals): saline-bolus versus control was 1.44 (1.09–1.90, $p=0.01$); HAS-bolus versus saline-bolus was 1.01 (0.78–1.29, $p=0.96$); bolus (HAS

or saline) versus control was 1.45 (1.13–1.86, $p=0.003$). Most fatalities occurred before 24 h (259; 87 %). The small number of fatalities occurring after 48 h provided no evidence that children in the control arm had excess delayed mortality [8].

In stratum B (children with severe hypotension and impaired perfusion) a total of 29 children, were enrolled with a median systolic blood pressure of 57 mmHg (interquartile range, 51–59). Thirteen were randomised to HAS of whom 9 (69 %) died and 16 randomised to saline and 9 died (56 %) died (relative risk of 48 h mortality with HAS bolus, 1.23; 95 % CI, 0.70–2.16; $p=0.45$).

Excess mortality associated with bolus arms compared to control was consistent across all pre-specified subgroups. Moreover, there was no difference in outcome between the two bolus arms (for all saline versus albumin comparisons), including the children in coma among whom we had hypothesised that albumin infusion might be neuroprotective [13]. At 28 days, HAS-saline-control mortality and neurological abnormality rates were 12 %, 12 % and 8.7 % ($p=0.004$ for bolus versus control) and 2.1 %, 1.3 %, 1.8 % ($p=0.85$ for bolus versus control); pulmonary oedema or raised intracranial pressure were reported in 30, 24 and 17 children, respectively.

20.3.2.6   Interpretation

Since the paper was published, there has been considerable debate through commentary papers, letters, at journal clubs and web-based discussion groups about the interpretation of the FEAST trial results [44–49]. The FEAST trial was even the subject of a pro/con debate at the IIC Meeting, Oxford July 2011. Many commentators on the FEAST trial have speculated on the reason for this surprising result, some raising concerns about the broad entry criteria. One of the major causes for concern was the definition of shock. In a subsequent letter to the NEJM, responding to nine of the numerous letters selected for publication, the investigating group presented new data on the relevance of the FEAST trial cohort to other definitions of shock [50]. These data are summarised in Table 20.2.

Table 20.2 Application of various shock definitions to FEAST admission data

	Shock definition	Mortality (%)			Absolute risk difference (95 % CI)
		Overall (all arms)	Bolus (saline or albumin)	No bolus (control)	
FEAST inclusion criteria	Impaired consciousness (prostration or coma) and/or respiratory distress plus ≥1 of: CRT >2 s; lower limb temperature gradient; weak pulse; HR >180 (<12 months), >160 (12 months to 5 years), >140 (>5 years)	297/3,141 (9.5 %)	221/2,097 (10.6 %)	76/1,044 (7.3 %)	3.3 % (1.2–5.3)
Paediatric shock scores					
ACCM-PALS	Pyrexia or hypothermia (core temperature >37.40 C or <36.0 °C) with either: hypotension (SBP <70 mmHg (<12 m); <80 mmHg (≥12 m) Or ≥2 of: altered mental status (agitation, prostration or inability to localize a painful stimulus); CRT >2 s; temperature gradient; bounding or weak pulse; HR >180 (<12 months), >160 (12 months to 5 years), >140 (>5 years)	194/2,030 (10 %)	150/1,389 (11 %)	44/641 (7 %)	3.9 % (1.4–6.5)
Surviving sepsis campaign	Lactate >5 mmol/l	216/1,159 (19 %)	157/764 (21 %)	59/395 (15 %)	5.6 % (1.1–10.1)
WHO	Presence of cold hands or feet with both CRT 4 or more seconds and a weak, fast pulse	27/65 (42 %)	24/50 (48 %)	3/15 (20 %)	28 % (3.4–52.5)

20.3.4 Implications for Policy

The results of the FEAST trial challenge the primacy of bolus resuscitation as a life-saving intervention in paediatric emergencies. The trial results showed that 89.4 % of those given boluses survived the first 48 h in hospital. But those given only maintenance fluids did better: 92.7 % of them survived. Put another way, this means that compared to maintenance fluids, boluses cause more than three children (3.3 %) to die out of every hundred treated.

These results are applicable to Africa, under the conditions that prevailed in the FEAST trial. There may be reasons why fluid boluses are harmful in such African children including specific range of pathogens and nutritional status. However, there should be further research to re-examine the physiological changes in shock and host response. For severe malaria, which was present in 57 % of the study population, the results are clear and should inform future policy. Hypovolaemic shock in severe malaria should not be resuscitated with boluses of isotonic crystalloid or colloids as outcome is worse than in children receiving maintenance fluids only. Children with signs of dehydration can safely be managed with dextrose/isotonic saline (0.9 %) maintenance fluids (4 ml/kg/h) since outcome is better than more aggressive rehydration or resuscitation with isotonic fluid boluses.

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