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ISBN 978-3-319-44973-9 ISBN 978-3-319-44975-3 (eBook)
DOI 10.1007/978-3-319-44975-3

Library of Congress Control Number: 2016956377

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Foreword

In the present volume the authors have addressed the complex relationships between human beings and water and food, which are the sources of energy and healthiness and the guarantor of our survival on Earth. The challenges the authors faced were certainly arduous especially due to the vastness of the problems inherent to the water and food supplies. Such problems are extremely diverse because of the wide variety of situations of the world population, which are dependent fundamentally on climate, level of instruction, economical means, and so on. The second notable obstacle, which the authors confronted also, is the general ignorance of this topic, which is very often neglected or underestimated in school's disciplines, so it happens that who must legislate on the topic is frequently not up to the issue to be addressed. The text of the volume encloses valuable information regarding water and food elements, from the point of view of microbial ecology, physiology, and nutrition. It also includes applied microbiology as well as water and food safety, ranging throughout the period of time of human existence from prehistory to our times, naturally favoring the years closer to more current experiences. It is of uttermost utility to agronomists, plant and animal scientists, as well as food scientists and food safety specialists, because they can possibly draw details in their studies and in their formulations of recipes which should be considered to satisfy the needs of any type of person, regardless of gender, age, socioeconomic status, geographical location or lifestyle, and each and every one. It is certainly useful for students of any age and also for the multitude of people who are interested in the topic, perhaps I would not say for the "insiders", a term which is now in vogue. It is also a pleasant reading for the ones, by now retired, to the study and to his evangelization, and also to revise the topics of its spent work. In summary, I welcome this volume covering concisely and efficiently a difficult and controversial subject for which we must spur researchers to study and to further spread and develop the concepts.

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Preface

Food safety is a multidisciplinary scientific discipline that is specifically designed to prevent foodborne illness to consumers. It is generally assumed to be an axiom by both nonprofessionals and professionals alike that the most developed countries, through their intricate and complex standards, formal trainings and inspections, are always capable of providing much safer food items and beverages to consumers as opposed to the lesser developed countries and regions of the world. Clearly, the available data regarding the morbidity and the mortality in different areas of the world confirms that in developing countries, the prevalence and the incidence of presumptive foodborne illness are much greater. However, other factors need to be taken into consideration in this overall picture: First of all, one of the key issues in developing countries appears to be the availability of safe drinking water, a key element in any food safety strategy. Second, the different availability of healthcare facilities, care providers, and medicines in different parts of the world makes the consequences of foodborne illness much more important and life threatening in lesser developed countries than in most developed countries. It would be therefore ethnocentric and rather simplistic to state that the margin of improvement in food safety is only directly proportional to the level of development of the society or to the level of complexity of any given national or international standard. Besides standards and regulations, humans as a whole have evolved and adapted different strategies to provide and to insure food and water safety according to their cultural and historical backgrounds. Our goal is to discuss and to compare these strategies in a cross-cultural and technical approach, according to the realities of different socio-economic, ethnical, and social heritages.

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Part I
Introduction

Chapter 1
The Complex Relationships Between Humans, Food, Water, and Hygiene

"Air, water, and food" are the most essential elements for the life of all animals. They represent the "physiological needs" which are indispensable for life and the most basic "metabolic requirements" for the survival of all animals, humans included [1]. At the second level of the Maslow's hierarchy of needs, one finds safety and security, which not only includes "personal and financial security" but also takes into account "health and well-being" and, especially, "safety against accidents/illness and their adverse impacts" [1]. Among all safety concerns, food safety seems to rank the highest in public perception in most developed countries, while for the rest of the less privileged world, improving drinking water supplies and achieving international water sanitation targets and most of all food security appear to remain the main physiological challenges facing the world's community [2, 3]. Worldwide, a general indicator of "inadequate levels of sanitation and hygiene" could be the incidence of diarrheal diseases, which ranks as 5th, among the 20 global leading causes of years of life lost[1] for both sexes combined. By comparison, the lack of protein energy and malnutrition ranks 20th [3]. At this present day and age, as the global "life expectancy at birth has increased (on average) by 6 years since 1990," as the "years of life lost due to diarrheal diseases has decreased by 40 % from the year 2000 to 2012," and as the "number of underweight children globally has declined from 160 million in 1990 to 99 million in 2012," new food-related health emergencies are on the rise such as child obesity. "In 2012 an estimated 44 million (6.7 %) of children under 5 years of age were overweight or obese world-wide" [3]. Food hygiene and food safety are therefore only one part of the overall picture concerning our complex relationships with food, water, and hygiene.

The current public opinion about different hazards in foods differs greatly from country to country. A difference is also seen with regard to the socioeconomic status

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[1] "Year of Life Lost (YLL) takes into account the age at which deaths occur by assigning greater statistical weight to deaths occurring at younger ages and lower statistical weight to deaths occurring at older ages" [3].

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and level of education of the persons surveyed. The 2010 Euro-barometer survey report on risks perception in the EU describes that "the majority of Europeans associate food and eating with enjoyment." "Those who are concerned about possible food-related risks tend to worry more about chemical contamination of food rather than bacterial contamination or health and nutrition issues." It was also noted that "most Europeans have confidence in national and European food safety agencies as information sources on possible risks associated with food" [4]. By comparison, according to the International Food Information Council (IFIC) Foundation, in their 2010 Food and Health Survey, Consumer Attitudes Toward Food Safety, Nutrition, and Health: "similar to previous years, close to half of Americans (47 %) rate themselves as confident in the safety of the U.S. food supply." By contrast, unlike the European survey, in the USA 44 % of the people rated "as the most important food safety issue today foodborne illness from bacteria" and as a second determining safety factor (39 %) "chemicals in foods" [5].

The differences between perceived risks and real risks in the food supply have been the subject of many publications and reports. In particular, in the 2003 annual report of the food safety agency of the Swiss Italian canton of Switzerland (Ticino), a comparison was shown based on the World Health Organization (WHO) data, which illustrates very clearly the differences between perceived food risks and real food risks by consumers [6] (Fig. 1.1).

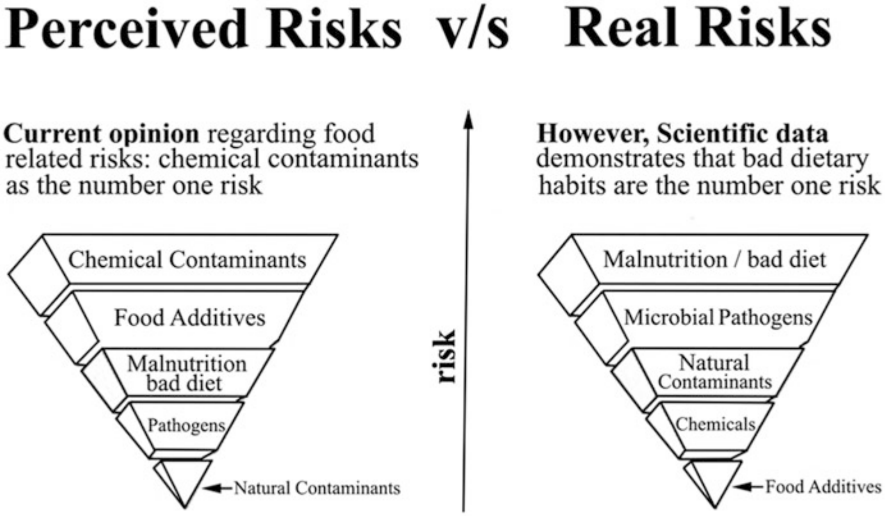

Fig. 1.1 Generally perceived risks such as chemical contaminants and food additives tend to be perceived more importantly than they actually are, while malnutrition/bad diet and microbial pathogens instead, actually represent much greater risks than perceived. *Source*: Adapted and translated from Laboratorio Cantonale Ticino and WHO [6]

To further complicate matters, not only does there seem to be wide gaps in the perception of the risks, which are related to food and water supplies, but there are also some very complex cultural and ethnical differences in what is considered to be a food item itself. Food is deeply imbedded in our cultural heritage and what is considered to be a delicacy in one part of the world or in one culture can easily be considered gross or unacceptable somewhere else. Most people would frown upon the prospect of eating food made from jellyfish, sea cucumbers, snails, frogs, arachnids, insects, earthworms, snakes, bats, and rodents, yet all of these food items are consumed somewhere in the world with some degree of safety.

Whether we are aware or not food has always had some components of ethnocentrism. We tend to trust the foods we know but are skeptical with what we are generally not familiar with. "Attributes such as color, size, price, or brand, information related to the product sourcing country"; "consumer knowledge of the product, a person's involvement and experience with the product category in question"; and information regarding the "country of origin" deeply influence our decision-making processes of what we buy and what we eat and drink. According to a study concerning customer ethnocentrism in regard to the information about the origin of the products, it was found that "consumer ethnocentrism increases with age, as well as when an individual comes from a lower than average income household" [7]. One possible explanation for such differences could very well be that people with a lower than average income have less opportunities to travel and therefore to confront themselves with other cultures and customs.

Ethnocentrism usually carries a negative connotation as it correlates to both prejudice and self-centering attitudes; however, ethnocentrism, to some reasonable extent, is also an adaptive strategy that favors the in-group versus the out-group. This concept can be demonstrated by the fact that "cultural evolution can lead to locally adaptive practices, reducing the prevalence of disease via hygienic behaviors, diet, patterns of food storage and preparation, medicinal traditions, mortuary practices," and other relevant behaviors. An example of this concept can be seen during "the first trimester of pregnancy" which "is a period of particular vulnerability to infection." A well-documented study of "disease-salient emotional states and ethnocentric attitudes" of pregnant women found that: "consistent with the predictions of the disease-threat model," a "favoritism toward the in-group peaks during the first trimester of pregnancy and decreases during the second and third trimesters" [8].

Chapter 2
A Brief History of Food, Food Safety, and Hygiene

Since food and water are such central physiological needs for life, humans must have had early in the evolution's path, probably through a combination of instinct behavior and trial and errors, some sort of adaptive strategy to avoid, as much as possible, foodborne diseases. Most likely, they were obtained through a combination of instinctual behavior and trial and errors. For instance, in the archeological and paleontological site of the Arago Cave in Tautavel in the department of Pyrénées-Orientales in southern France, scientists found that "sometime between 450,000 and 550,000 years ago," prehistoric humans (*Homo erectus*) successfully hunted several large herbivores ("elk, reindeer, and fallow deer"; "bison, musk ox, argali, and tahr"; "chamois"; and "horses and rhinoceroses"). It was discovered that the bone remains "show markings made by prehistoric tools" not only to remove the flesh from the bones but also that "all of the bones have been fractured" to extract the "bone marrow" [9]. We now know through our current scientific knowledge that the bone marrow, until it is extracted from its bone cover and matrix, remains naturally protected from contamination caused by spoilage and/or pathogenic microorganisms. This activity can therefore be viewed as a rudimentary form of food conservation.

The subsequent step of the "control of fire by early humans" is still rather controversial. While many scholars agree that *Homo erectus* began to domesticate fire some 400,000 years ago, claims of much earlier control of fire through different findings in Africa, Middle East, and Asia are gaining "increasing scientific support" [10]. Nevertheless, in the archeological site of Terra Amata near Nice by the Mediterranean Sea, archeologist found that hominids 380,000 BCE lived in huts by the beach and that those "habitations included vestiges which suggested that in the center of each hut" there "was a fireplace, with ashes showing that the inhabitants had domesticated fire." "These signs of fire," along with other archeological sites, "are the earliest evidence of the domestication of fire known in Europe" [11]. No one knows, however, if fire was only used for warmth and protection, or it was also used for some sort of rudimentary roasting or cooking. Ward Nicholson, citing the

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works of Wu and Lin in 1983, Rowley-Conwy in 1993, and Megarry in 1995, “it does seem likely that at least by 230,000–460,000 years ago humans were using fire in the cave, and given scorching patterns around the teeth and skulls of some animal remains, it does appear the hominids may have done this to cook the brains (not an uncommon practice among hunting-gathering peoples today)” [12].

According to the “cooking hypothesis” by primatologist Richard Wrangham, “cooking had profound evolutionary effect because it increased food efficiency which allowed human ancestors to spend less time foraging, chewing, and digesting.” He also claims that *Homo erectus* “developed via a smaller, more efficient digestive tract which freed up energy to enable larger brain growth” [13]. Critics of this hypothesis however argue that our “human ancestors scavenged carcasses for high-quality food that preceded the evolutionary shift to smaller guts and larger brains” [14]. Notwithstanding, it might be worth pointing out, in regard to the possible dietary habits of the gathering and hunting hominid societies during the Pleistocene, many scholars now feel that “early researchers in cultural anthropology overemphasized the importance of hunting to the group’s survival, giving the impression that hunting provided most of their food and stressing the male’s vital job as hunter.” In this respect, Lee and De Vore (1968), cited by Weiss and Mann, remind us that “we have found that hunting might not have been that important, and that the fruits, vegetables, nuts, birds and bird’s eggs, insects and insect grubs, frogs and other small vertebrates, and small mammals gathered were the main part of their diet” [15].

These cultural anthropological observations prompt us at least to two considerations: the first is that these practices seem to be consistent with what we now observe with the few isolated and “primitive” societies we have left today in this world. The second is that given what we now know to be true about the probable microbiological and parasite profiles of those foods of animal origin (wild birds, small vertebrate, and small mammals (including rodents)), we can easily speculate that thorough heating (or cooking) must have been a necessary and an almost indispensable adaptive behavior early on, in order to allow the perpetuation of these eating habits over such a long period of time.

From an historical point of view, “it is extremely difficult to pinpoint the precise beginning of human awareness of the presence and role of microorganisms in foods, the available evidence indicates that this knowledge preceded the establishment of bacteriology or microbiology as a science” [16]. Our knowledge is not only limited to what we can understand but especially to what we can see. It is therefore not surprising that the first organisms that we could easily identify as “parasites” were worms, since many parasitic worms can be big enough to be seen with the naked eye. The first written records on parasitic worms come to us from the “Ebers Papyrus” of ancient Egypt (ca. 1500 BCE), where medical practitioners gave great importance to parasitic worms, against which they described several recipes and exorcisms for their attenuation or eradication. Interestingly, their etiological concept was similar to the theory “spontaneous generation” in ancient Greece, which can be described as the “diseases generate worms and not worms generate diseases” [17].

The transition from gathering and hunting societies of the Paleolithic to the new stones or "food-producing period" of the Neolithic is generally believed to have begun—based on the known archeological and paleontological records—about 8,000–10,000 years ago. There seems to be an agreement that "the problems of spoilage and food poisoning were encountered early in this period. With the advent of prepared foods, the problems of disease transmission by foods and of faster spoilage caused by improper storage made their appearance" [16]. This concept seems to be reasonable, not only because of the greater complexities which are necessary for food-producing activities as opposed to gathering and hunting but also because the size of human groups tends to grow in accordance to the availability of food resources. Consequently, the establishment of permanent settlements and the resulting greater proximities and densities of populations would also make it more likely for pests, pathogens, and parasites to contaminate and infect humans.

Human domestication of food plants and cereals in particular, began approximately 17,000 years ago and naturally preceded the domestication of large herbivores. Around 9,000–10,000 years ago, important plant and animal species such as sheep (*Ovis aries*), goats (*Capra aegagrus*), cattle (*Bos taurus*), and wild boar (*Sus scrofa*) (~7,000 years ago) were domesticated by humans. Despite their obvious contributions in the production of milk, meat, wool, and skins, sheep and goats were also traditionally used in ritual or religious sacrifices [18]. Ritualistic sacrificial offering of animals or animal products (such as clarified butter) is well documented in most traditions and religions, and they are designed to appease the Deities and for protection of the group against natural disasters, famines, diseases, and other adverse natural effects [19]. Historically, tradition and religious beliefs seem to provide the first line of teachings to humans about hygiene and epidemics, long before any true scientific approach was ever developed.

For instance, in the ancient Hebraic religious writings, there are many references that describe the paramount importance that the Israelites must have given to the roles of insects as propagators or agents of diseases. Flies were seen as the physical expression of the prince of the devil Beelzebub (*Ba' al zebûb*), and intestinal worms, rodents, and other pests were all considered dangerous because of their inherent "impure" nature. Plagues and diseases were believed to be "divine castigations." However, one important principle of disease prevention was already recognized at time, which was to actively isolate the sick or "impure" from the healthy to prevent transmission and to require a "purifying bath" before a patient could return to the community [20]. The religious teachings of the Torah or Pentateuch not only recognized the risks of contagion of pests to human and between humans but also warned against the inherent dangers caused by the sick to the crops. In Numbers V, 1–3: "the LORD said to Moses: Order the Israelites to expel from camp every leper, and everyone suffering from a discharge, and everyone who has become unclean by contact with a corpse. Male and female alike, you shall compel them to go out of the camp; they are not to defile the camp in which I dwell" [21].

In India, in the sacred Hindu book of the Atharvaveda (1200–1000 BCE), diseases are also believed to be the "actions of demons or by foreign malefic substances which produced some sort of, not personified, fluid or ether that penetrates the human and prostrates him.” We can thereby state that “in the ancient Indian medicine, we are faced, for the first time, with a rational concept: the one of an external etiology for contagious diseases” [22]. Also in the far East, ancient Chinese “not only understood that certain diseases, like smallpox, are contagious but realized that the ones who had been able to overcome smallpox itself would no longer be susceptible, so they thought to purposely contaminate the children in order to provoke in them a disease that would protect them as adults.” This technique known as *variolation* consists in utilizing the “dried infective crust of smallpox patients and inoculate it in small quantities in the nostrils of children by means of a stick or blown through a small bamboo twig.” This can be considered to be the very “first immunological practice that for centuries has remained unfortunately circumscribed only to the far East” [23]. In addition, in China, ca. 1000 BCE, some of the earliest records of modern food preservation are also documented including “drying, smoking, salting, and spicing.” Interestingly, “wine, converted to vinegar, is also used for food preservation” [24].

Spontaneous fermentations have probably been observed since the beginning of the first stable human settlements in their first attempts to store fermentable food. It seems like Neolithic humans might have somehow managed to make some sort of beer and wine very early; however, archeological evidence in this regard seems only to appear around 4000 BCE [18]. The first alcoholic fermentation was probably spontaneous or accidental, and it certainly involved wild yeasts typically occurring on the surface of the vegetable materials and in the environment. “The term fermentation is derived from the Latin verb *fervere*, to boil, thus describing the appearance of the action of yeasts on extracts of fruits or malted grains” [25]. Mature grapes, fruits, must, and juices have very complex microflora, including “yeasts, lactic and acetic acid bacteria, and molds” [26]. Once the alcohol is converted from simple sugars, unless care is taken to prevent oxidation, acetic acid bacteria will eventually oxidize ethanol to acetic acid, thereby producing vinegar. A similar sequential process can also be observed in the fermentation of malted cereals and fermented rice.

Other very old microbial food processes are the production of sour milk, yogurt, and sour cream. These products may have originated from some successful attempts to conserve dairy products in stable human settlements around 5000 BCE [18]. Although, at least “theoretically, milk that is secreted to the udder of a healthy cow (or other healthy mammal) should be free of microorganisms.” “Freshly drawn milk is generally not free of microorganisms. Numbers of several hundred to several thousand colony forming units per mL are often found in freshly drawn milk, and they represent the movement up the teat canal of some and the presence of others at the lower ends of teats” [27]. It can therefore be assumed that in the early stages, the naturally present microbes and the lactic acid bacteria, usually present in fresh milk, when stored in the warmer dwellings of the Neolithic, initiated spontaneous lactic acid fermentation, thereby catabolizing “lactose to lactic acid and lesser amounts of acetic acid in the critical step of lowering the pH” of the substrate [28].

One must realize that by “spontaneous,” we usually mean now the natural evolution of any endogenous microbes in a substrate, as opposed to the inoculation of a substrate with microbes. It should not be confused with the theory of “spontaneous generation." This theory, which was considered axiomatic since ancient Egypt, remained a doctrine for millennia, causing many polemical debates, until it was finally abandoned by modern microbiology. During the ancient Greek civilization, one of the most respected Western philosophers of all times, Aristotle, believed "there are animals that grow spontaneously inside other animals" [29]. The theory of spontaneous generation was based on what people could see with their own eyes: usually "maggots originate from rotting meat." Notwithstanding to the fact that during the Roman civilization, unlike during Greeks times, Roman scholars had "perfectly understood some aspects of the contagious nature of some diseases," "describing pests and pestilent sites, contaminations of herds, diseases that would affect contemporarily group of people and their dogs, infections of wounds from fly's maggots, and the diffusion of scabies among animals." In general, however, in most concepts of infective pathology, Romans "limited themselves to repeat" and to "translate from Greek to Latin" what "Greeks had already written before them" [30].

Although the nature and especially the origin of the microbial world would not be understood for many years to come, by recognizing the existence of pestilent sites (like swamps) and by understanding somehow the causal relationship between disease and contagion, some effective preventive measures were taken long before the "germ theory of the diseases" could be understood and accepted. Around the year 900 AC, "the leader of the Byzantine Empire, Emperor Leo VI, forbids the eating of blood sausage because of its association with a fatal food poisoning now presumed to be botulism." Interestingly, the actual term "botulism," which originates from the Latin word for sausage *botulus*, will "come into use at the beginning of the nineteenth century because of the association of the disease with eating sausage." Also in 1403, as the "bubonic plague strikes Venice, in an attempt to protect its citizens, the city establishes a policy that no one can enter the city until a certain waiting period has passed." The waiting period is 40 days (in Italian *quaranta*) giving origin to the term still used today: *quarantine* [24].

Microorganisms are separate living entities, and they are not the by-product of decaying higher organisms. Many more scientists than the ones mentioned here contributed over centuries to this gradual conceptual understanding. Generally, the most cited is Girolamo Fracastoro who in 1546 in the *De contagione* suggests that "infections and epidemics are caused by *seminaria*, or seeds of disease." Seminaria were considered living or "exhalations from onions that causes tearing." Fracastoro also "suggests three modes of transmission: direct contact, fomites (inanimate objects), and contagion at a distance (through the air)." In 1658, Athanasius Kircher in *Scrutinium Physico-Medicum Contagiosae Luis, quae dicitur Pestis* proposes that "small, imperceptible living bodies are the sources of contagion and penetrate clothing, ropes, linen, sheets, and anything else that has small pores." However, "because Kircher's writings are ambiguous, often incomprehensible, most students of his work regard them as being of little if any value." In 1668, Francesco Redi "places meat into two dishes, leaving one open to the air and the other one covered. He repeats the experiment leaving one dish open and the other covered with gauze. In both experiments, maggots appear only in the uncovered dishes." Not surprisingly, however, "few people believe that life cannot be spontaneously generated" [24].

The first documented record of any direct observation of the microbial world comes from Antonie van Leeuwenhoek, who observes and measures in his microscope molds (1673); protozoa, green algae, and red blood cells (1674); bacteria (1676); spermatozoa (1677); yeasts (1680); and oral bacteria (1683). These observations can be assigned with a high degree of historical precision because van Leeuwenhoek systematically "sends more than 200 letters describing many observations with the use of his microscopes" to the Royal Society of London. As the debates on the theory of spontaneous generation continues, Lazzaro Spallanzani, in 1765, repeats the experiments of John Turberville Needham in which "meat infusions were boiled in corked glass tubes but improves upon them by melting the tops in a flame to seal the tubes. He concludes that microorganisms do not arise spontaneously if the infusions are heated for a sufficiently long time." He is credited to have been the first person to "suggests that food can be preserved by sealing in airtight containers" [24].

Spallanzani's observations are not only important to the advances of pure science but also to the advancement of food technology. His experiments are replicated by a very dynamic confectioner with no formal scientific training, Francois Nicolas Appert, who in 1804, "after several years of experimentation, opens a factory for preserving food in glass bottles using a heating process that he invents." Then in 1808, "he reports experiments performed over two decades that show food can be successfully preserved through a heating process." In 1810, he publishes *Le livre de tous les ménages, ou l'art de conserver, pendant plusieurs années, toutes les substances animales et végétales*, where he explains "his method for preserving food in bottles" during many years [24]. Appert is credited to be the "inventor of airtight food preservation" and the "father of canning" [31]. However, it should be pointed out that Appert's "canning" was carried out only in glass containers until 1819, when "Peter Durant replaces Nicolas Appert's glass containers with steel cans," and in 1839, when Charles Mitchell adds "a thin layer of tin to line steel cans" [24]. Although Appert's discoveries provide the basic rationale for subsequent findings in food preservation by heat, "appertization" should not be confused with pasteurization, which comes later in time, because pasteurization employs a gentler heating process than "appertization" [31].

In 1838, Charles Cagniard de la Tour, "based on microscopic observations of budding and growing brewer's yeast, concludes that yeast is a living organism that might cause fermentation." His findings are in agreement with the colleagues Pierre Turpin, Theodor Schwann, and Friedrich Kützing, who are however ridiculed in 1839, in "a humorous article" which appear in a "journal edited by Friedrich Wohler, Justus von Liebig, Jean-Baptiste Dumas, and Thomas Graham." Since these editors were eminent chemists at the time, "this article may have significantly delayed the acceptance of Cagniard de la Tour's conclusions about fermentation." In 1840, as part of the long debate surrounding the germ theory of disease versus spontaneous generation, Friedrich Gustav Jakob Henle, who was "one of Robert Koch's teachers at the University of Göttingen," proposes in his book *Pathologische Untersuchungen* the existence of "living entity that reproduces itself and possibly could be grown outside the body" [24].

In 1846, “cholera pandemic in India spreads across Europe, reaching England,” and in 1849, “John Snow presents his first observations on the transmission of the disease cholera through drinking water.” “Snow is awarded a prize of 30,000 francs.” In 1854, “Filippo Pacini describes the cholera bacillus and names it *Vibrio cholera*,” and 1 year later, John Snow publishes *On the Communication of Cholera by Impure Thames Water* and “provides quantitative data on the spread of the disease through water contaminated by sewage. In one of the earliest thorough epidemiological studies, he traces one outbreak in London to the Broad Street water pump (now Broadwick Street in Soho), showing it to be contaminated by raw sewage.” In 1856, William Budd suggests that the fecal-water route is also responsible for the transmission of typhoid fever through contaminated drinking water. Although William Budd rightly recommends the use of different disinfectants in water to control the infection, “chlorine water, chloride of lime, and carbolic acid,” the first suggestion to use chlorine (formerly called oxymuriatic acid) “for the sanitation of drinking water” is however assigned independently to Guyton de Morveau and William Cruikshank in 1800 [24]. In this regard, it must be pointed out that presently in most developing countries, “chlorination is the principal disinfectant used in water treatment, with risks associated with chlorination byproducts being considered far less significant compared to consequences of waterborne disease” [32].

Louis Pasteur’s contribution to the advancement of microbiology and to the development of modern food processes cannot be underestimated. The process named after this French scientist differs from appertization. During the pasteurization of canned food, the perishable food items contained in the cans are subjected to a much milder “process which permits a limited shelf-life if kept cold,” while in the process of appertization, one achieves “commercial sterility,” thus obtaining “shelf stable” products which can be stored at room temperature [33]. Louis Pasteur’s contributions to science are not limited to his most famous experiments with swan neck flasks to disprove the theory of spontaneous generation but largely mark “the birth of industrial microbiology,” when in 1860 and on his subsequent work, he elegantly and “finally demonstrates beyond doubt that alcoholic fermentation in beer and wine production was the result of microbial activity, rather than being a chemical process” [34].

Pasteur makes many fundamental ingenious observations in many areas of microbiology. He is credited to be the first scientist to describe some important aspects of “butyric acid fermentation” such as the “fermenting bacteria live without free oxygen” and that in this process “oxygen inhibits fermentation,” thereby being the first to apply the term *anaérobies* for anaerobes. Pasteur confirms Friedrich Kützing observations in 1837 that aerobic bacteria are the “microscopic organisms smaller than yeast” that live on “the film known as the mother of vinegar,” which is “responsible for the conversion of ethanol to acetic acid.” Pasteur also demonstrated that lactic acid bacteria convert lactose to lactic acid. Despite his many contributions and brilliant achievements, there are also some controversies surrounding some of his premature recognitions, which remain in the historical records. For instance, when in 1861, Pasteur is awarded by the Paris Academy of Sciences the “Alhumpert Prize” with a premium of 2,500 francs, for “throwing new light on the question of spontaneous generation," "the actual conclusion of the controversy" will only come later with the experiments of John Tyndall (1876) and Ferdinand Cohn (1875, 1876), who elucidate the important survival mechanism of "heat resistance endospores," in endospore-forming bacteria [24].

Bacterial endospores still can represent a serious threat to the safety of food items today because they are able to survive most conventional cooking and heat treatments. The first drawings of bacterial endospores are attributed to Maximilian Perty in 1852, but he did not assign any function to them. In 1872, Henry Charlton Bastian "revives the argument about spontaneous generation of life" by reporting an experiment where "he boils acid urine for several minutes and then neutralizes it with a potash solution prepared with distilled water he believes to be sterile, but possibly introducing contaminating bacteria." We can assume that most probably these contaminants were endospore-forming bacteria of the genus *Bacillus*. In response, between the years 1876 and 1877, after many trials and many errors with boiled hay infusions (which naturally contains bacterial endospores), John Tyndall "develops a procedure of several cycles of heating, incubating, and reheating his culture tubes that results in sterilization even of the heat-stable phase. This technique, known as "tyndallization," is used as a means to achieve sterility without using steam under pressure." Ferdinand Cohn confirms in 1877 "that *Bacillus* species found in hay have heat-resistant endospores." Surprisingly, however, in 1869 while studying the silkworm disease, Louis Pasteur had already noted that "some bacteria have dormant stages, refractile bodies" which are "more resistant to heat than actively growing cells" but failed to recognize them as bacterial endospores [24].

Louis Pasteur should also be remembered not only for his discoveries in microbiology and immunology but also for having positively inspired other key scientists. Among others were Joseph Lister, who is considered to be the father of the "practice of antiseptic surgery," and Robert Koch, proponent of the first experimental demonstration to identify an infective agent. The rationale behind the "Koch's postulate" actually relies on the logical criteria, originally proposed but not experimented by Edwin Klebs in 1877 [24]. The experimental evidences leading to the formulations of the Koch's postulates were "presented in 1883 by renown German bacteriologist, Robert Koch," with a later addition of a fourth rule, by American phytopathologist Erwin Frink Smith, in 1905 [35].

These rules can be summarized as follows:

1. "The suspected causal organism must be constantly associated with the disease" [35].
2. "The suspected causal organism must be isolated from an infected plant and grown in pure culture" [35].
3. "When a healthy susceptible host is inoculated with the pathogen from pure culture, symptoms of the original disease must develop" [35].
4. "The same pathogen must be re-isolated from plants infected under experimental conditions" [35].

With our current knowledge, it must be pointed out that the Koch and Smith protocols for the determination of the causal relationship between pathogen and disease are only applicable to viable and culturable microorganisms. Some bacteria, for instance, are able to go in a sort of "dormant state" thereby remaining "viable and potentially virulent but nonculturable by standard methods" [24]. Currently, with more advanced understandings and technological methods in virology and molecular microbiology, we are able to go "beyond the Koch's postulates" and understand some of the "false-negative" conclusions we might have obtained in the past in the lab [36].

In this modern era, in what we consider to be the most "developed parts of the world," we generally take for granted our very high standards of living and our complex norms and regulations regarding housing, personal, and manufacturing sanitation and hygiene. As we have seen, in the prescientific era, tradition and religions provided some sets of rules and commonly accepted practices, which were promoting some basic hygiene criteria. Soaps or soap-like substances and vinegar [37], for instance, have been known and used for cleaning for several thousand years and all over the world [38]. Disinfectants, however, have been an important aspect of our good hygienic practices for a much shorter time. Surprisingly, although alcohol distillation has been a practice by Arab chemists since 900 AC, and in vogue the late eleventh century by Europeans, the beginning of alcohol being used as disinfectant does not seem to appear until much more recent times. Hand disinfection techniques we use in food production today originate from the earliest medical antiseptic practices, which were first "met with strong opposition" from the medical community at the time [24].

Historically, when in 1842, Oliver Wendell Holmes suggests to disinfect the hands after "postmortem examinations or attending patients with puerperal fever," his ideas were generally discarded by most scholars at the time, with the notable exception of Ignaz Philipp Semmelweis, who, in 1846, "introduces the use of antiseptic (hypochlorite solution) in his work on prevention of the spread of puerperal fever" [24]. Hypochlorite solutions like common bleach are still considered, along with alcohol, as some of the most classical and most commonly used disinfectants, which are still effective against most pathogenic microorganisms today [38]. Unfortunately, however, when as early as 1861, Semmelweis "publishes *Die Aetiologie, der Begriff und die Prophylaxis des Kindbettfiebers*" which "requires medical students to wash their hands in chloride of lime upon leaving the autopsy room and before examining patients in the maternity ward," his ideas are not only refused but are strongly "opposed by the director of the hospital and other physicians," leading him to eventually become so "disturbed because his work goes unrecognized," to die insane, in 1865 at the aged 47, after being forcibly recovered in a mental asylum. Sadly and ironically, "his careful work survives and is recognized as an important contribution to antiseptic medical practice" [24].

Part II
The Elements of Complexity

Chapter 3
Food Microbiology Seen from Different Angles

In early microbiology from the mid-1800s "until the late 1800s, almost all emphasis was placed on the pathogen." The pathogen alone was considered to be "the sole cause of disease." The earliest studies focusing on the suscepts and on the environmental conditions in relation to the pathogens come from phytopathology. The original plant pathology's "triad concept of disease" states that plant diseases can be described as equilateral triangle constituted by a "suscept, the pathogen, and an environment favorable for disease development." "Only when all three factors are present is the triangle formed, i.e. disease develops" [40]. In phytopathology the disease triangle is no longer considered to be equilateral. Models are proposed for the disease triangle to have unequal sides and angles in order to account for importance of disease resistance and/or to take into consideration relative differences in terms of the pathogen's virulence. Alternatively, it is represented as "a disease pyramid or tetrahedron, in order to allow the addition of a fourth causal factor of disease" such as a vector, or the human intervention in the agro-ecosystem. Other models can describe a "disease cone," or "right angle prism," or "a series of sequentially stacked triangles," to incorporate also the "expansion of disease intensity through time." Although the conceptual models for plant diseases in "triangular relationships are unique to phytopathology," they provide a valuable rationale to examine all disease relationships from different angles, and not just from the pathogen's side [41].

In this respect, it must be first recognized that in food microbiology, foods are generally derived not only from plants but also from animal tissues. Animals, unlike plants in their interactions with microbes, pose more "thermal storage capacity" which alone can influence the associated animal microbial community or microbiomes. Unlike plants, animals can also "escape from an inhospitable environment," and most animals, especially mammals, are equipped with a "sophisticated immune system" [41]. However, despite these profound differences between animals and plants with regard to pathogenesis, both "plants and animals that serve as food sources" have in common "to have evolved mechanisms of defense against the invasion

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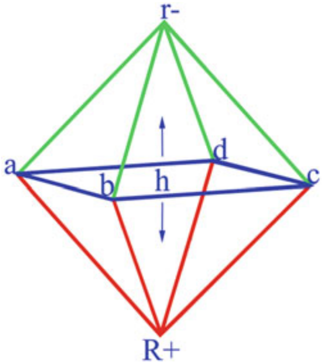
and proliferation of microorganisms." These defense mechanisms that are an "inherent part of the tissues are referred to as intrinsic parameters." Since some of the intrinsic parameters do "remain in effect in fresh foods," they must be considered important for food conservation such as "pH, moisture content, oxidation–reduction potential, nutrient content, antimicrobial constituents and biological structures" [42]. In addition to intrinsic parameters, there are also "extrinsic parameters of foods," which are characterized by being "not substrate dependent" and can be influenced by human decisions. These extrinsic parameters are "properties of the storage environment that affect both the foods and their microorganisms." Among others, "of greatest importance to the welfare of foodborne organisms are 'temperature of storage, relative humidity of environment, presence and concentration of gases, presence and activities of other microorganisms'" [43].

In addition, the mammalian's immune system provides many powerful lines of defense against many microorganisms. The term immunity "refers to a state of acquired or innate resistance or protection from a pathogenic microorganism or its products or from the effect of toxic substances" [44]. Immunology comprises many aspects and conditions that protect mammalians and that can only be briefly mentioned here. "Passive immunity" is usually acquired very early in life, for instance, through "the transfer of IgG antibodies across the placenta from mother to fetus" and again "through the ingestion of colostrum-containing antibodies" by newborn. Passive immunity is only designed to give some "temporary protection" to the recipient, but "no immunological memory is established." "Innate immunity" or natural immunity is instead usually present for the whole life since birth. Innate immunity "is designed to protect the host from injury or infection without previous contact with the infectious agent." "It is attributable to physical, chemical, and molecular defenses that prevent contact with antigens in a nonspecific manner." Innate immunity also "does not involve immunological memory" and "includes such factors as protection by the skin, mucous membranes, lysozyme in tears, stomach acid," and numerous other "antibacterial molecules" such as "complement and cytokines." This also includes cells such as "phagocytes and natural killers," which are some of "the key participants" and the first line of defense or earlier "barriers to infection" [45].

"Active immunity" is indeed acquired. Active immunity is the result of the "protection attained as a consequence of clinical or subclinical infection or deliberate immunization with an infectious agent or its products." It is therefore a "type of adaptive immunity in which lymphocytes are activated in response to a foreign antigen to which they have been exposed" [45]. The "protection from an infectious disease" due to adaptive immunity, when it does occur, is mediated by "B and T lymphocytes following exposure to specific antigen." Adaptive immunity "is characterized by specificity, immunological memory, and self/non-self recognition" [46]. Unfortunately, because of the complexities of the microbe-immune-system interactions, not all of exposures can possibly result in long-lasting protective immunity. Moreover, sometimes the memory and the responsiveness of the immune system can also "backfire." In fact, these are many instances when almost "paradoxically" is the immune system itself in "response to a pathogenic microorganism, rather than the microbe," which "induce injury to host tissues" [47].

It is therefore a daunting challenge to attempt to describe water and foodborne diseases in simple terms, mostly because so many factors come into play. In food microbiology besides the three basic elements of the disease triangle: suscept, pathogen, and favorable environmental conditions, we must also consider the host innate adaptive immunity and include both the dynamics and the presence of the human microbiome. Certainly, the inoculum size is always of paramount importance, but above all, we must never forget that the human animal can actually escape most, if not all, water- and foodborne diseases. Escape from water- and foodborne diseases can be achieved in many ways: the simplest is to avoid the water or the food one does not trust, but this can be impracticable sometimes. Another way would be to process the water or the food item with an acceptable heat treatment, either according to sciences or also according to cultures and traditions. Most importantly, escape can usually best be achieved through good education in food safety, sanitation, and hygiene and by applying correct microbiological risks management.

Fig. 3.1 Elements and points of view of diseases in food microbiology



Source: Adapted and inspired from the illustrations and text by Francl [41]

In this model, the elements of food safety are organized in an octahedron as follows:

In the center of a regular octahedron, we first place the human host (h).
At the corners in the horizontal plane of the octahedron, we have:

- The pathogen (a)
- The environmental conditions (b)
- The host innate and adaptive immunity (c)
- The host-specific microbiomes (d)

The relative strengths and weaknesses of the elements (a), (b), (c), and (d) and the nutritional status of the host (h) result in the host being repositioned inside a modified octahedron.

The vertical directions of the host (h) inside the octahedron also account for:

- Top direction: a relative decrease of the microbiological risks (r−)
- Bottom direction: a relative increase of the microbiological risks (R+)

The results of strength and weakness in the horizontal plane and the host (h) microbial risks management on the vertical plane determine whether the host (h) moves up in a comfortable area of improved microbiological food safety or down in a more dangerous area of greater microbiological risks.

This model is applicable to single and combined activities of our food and water supply chains from "farm to fork" [48].

Chapter 4
The Viruses

In order to accurately attempt to describe the microbial world, one should probably start from those considered to be probably the oldest, the smallest, and certainly the most numerous and diverse of all microbes: the viruses. Viruses are primarily known for being very small and elusive pathogens and generally generate a "public sentiment of fear and loathing." In fact, the word itself *virus* literally comes from the Latin word "poison" [49]. Since most viruses are small enough to pass "through porcelain filters" while retaining their virulence and cannot be grown on classical bacterial media, they were long thought to be chemical substances and not microbial entities [24]. Interestingly enough, although "the tree of life" excludes viruses and phages, "every living organism" in all ecosystems of this planet is "infected by at least one, and usually many, virus." It can therefore be asserted that all living organisms "are linked by their universal ability to be infected by viruses" [49].

Understandably, the actual numbers of viruses and phages on Earth can only be approximated. In this aspect, some estimates provide at least "a sense of scale," giving rise to some astronomical numbers. For instance, based on what is known about microbial oceanic communities, there should be at least "10^{31} viral particles on Earth" that can either be seen or counted. This estimate is extrapolated from data from "oceanic environments" where viruses and phages outnumber prokaryotes by a ratio of 10:1 [49]. Phages only infect prokaryotes, and it is estimated that they "kill up to one-third of marine bacteria every day," representing a likely "keystone predator" that keep "fast-growing bacterial populations" under check [50]. It must be also pointed out that the estimated number of viral particles on Earth does not take into consideration all viruses that are "fully or partially integrated into host genomes," which are also some significant, yet less visible entity. For instance, "8 % of the human genome appears to be derived from previous viral integration events." Current estimates of the biomass and total size of the viral biomes are extraordinary. According to Greene and Reid, based on the estimates of Shuttle in 2005: "even considering a lower bound of 10^{31} viral particles, each of which contains about 0.2 fg of carbon" or (2×10^{-16} g) "and is about 100 nm long" or (1×10^{-7} m), "yields

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the viral equivalent of almost 200 million blue whales." Moreover, if "stretched end to end," viral "particles would span about 25 million light years or about 250 times the distance across our own galaxy" [49].

A true estimate of the number of "viral species" on Earth cannot really be achieved, not only for the numbers involved but also because viruses have inherently "high mutation rates and genome plasticity" which elude a "traditional species concept." Viruses are not only the most numerous and "most diverse collection of biological entities on the planet," but they also show the greatest morphological and molecular variations "in size, shape, genome size, nucleic acid composition, replication strategy, host, genome content, and function." If they could be represented in species, they would represent, by far, the greatest possible number of "species" on Earth, with close to "1.7 billion species." Viruses, despite their bad reputation and their known roles in exerting a major "selective pressure on all life forms," are excellent "genetic engineers," as they are capable of introducing in their hosts new "pathogenic and beneficial genetic traits," through "horizontal gene transfer." Viruses can and do "shape the evolution of other organisms" by driving the "immune evolution" through pathogenicity, not only they are just infecting and killing some bacterial and other living cells. Viruses actually "increase their own reproductive fitness by boosting host fitness"; they are also known to "influence host behavior to spread" and to "participate in multitrophic symbioses." Paradoxically, the smallest known organisms on Earth, at a larger scale, can "exert indirect evolutionary pressures on ecosystems" and are also considered to be "major drivers of global biochemical cycles." Since present viruses are obligatory cellular parasites, the origin of viruses poses a similar dilemma to the one of which came first: the chicken or the egg? From an evolutionary standpoint, although the exact evolutionary origin of virus is still uncertain, and taking in consideration that "to date, no self-replicating or completely free-living virus has been identified," it seems plausible that "some viruses and their genes could have come from cells," while "other viruses could have given rise to cells" and "subsequently lost the ability to self-replicate," in a similar type of reductive evolutionary mechanism, comparable to the one generally accepted by scholars with "endosymbiotic bacteria" [49].

Surprisingly, despite common and customary negative reputation, astronomic numbers, genetic diversity, and high incidences and prevalence in every conceivable ecosystem, only a handful of virus "species" can be considered to be actual water- and foodborne pathogens [51, 52].

Chapter 5
The Bacteria

Taxonomically, prokaryotes are divided into two distinct domains: Archaea (formerly known as Archaebacteria) and Bacteria. Archaea are no longer considered the ancient and primitive predecessor of bacteria acknowledged mainly for the few extremophiles [24]. The importance of archaeal and bacterial communities in many ecological habitats and ecosystems is now widely accepted. We should realize, however, that "life not only began with microorganisms, the continued existence of life on Earth totally relies on the inconspicuous microbe." In fact, "more photosynthesis is carried out by microbes than by green plants." If one excludes cellulose of plants, "microbes constitute approximately 90 % of the biomass of the whole biosphere" [36]. Reid, citing the estimates by Whitman et al. in 1998, reminds that planet "Earth is home to an estimated 10^{30} microbes—about half of Earth's biomass." In the microbial world, "there are literally millions of different kinds of microbes living in complex, interdependent communities in every imaginable habitat." If we explore our possibilities to gain some understanding about these communities, we must first realize that it is very "difficult to simulate the complexity of natural systems". In addition, we must also keep in mind that "there are too many different microbial assemblages in too many different environments to hope that even a small fraction of them could ever be brought into culture in the laboratory" [53]. Lastly, we must acknowledge that we are usually limited to "study thoroughly in the lab at best one percent (1 %) of known bacteria and archaea, as the culture requirements are either unknown or impractical to implement for the vast majority of prokaryotic microbes" [54].

It is generally accepted that in "many microbial ecosystems, the functionally active unit is not a single species or population but a consortium of two or more types of organisms living in close symbiotic association." However, it must be noted that even within the same species of a given consortium, the genetic diversity is usually by far greater than the ones of "plants and animals." Bacteria not only interact with bacteriophages, but they are also able to independently transfer genetic information through conjugation either among members of the same species or other

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species [55]. Bacterial communities should be distinguished from microbial assemblages because in communities "members interact," while in assemblages "members merely coexist." Bacterial species and their consortia either can be "restricted to specific habitat types and geographical locations" or can be considered to be "cosmopolitan," thereby inhabiting either different habitats or different geographical locations. In terms of individual and collective behavior, bacteria can either be considered "free living" or "planktonic" or can dwell on many different substrates, surfaces, and sediments in communities known as "biofilms." Bacteria can also colonize different substrates and different tissues to form some "thick accumulations of interacting cells," which are known as "microbial mats" [56].

It is important to realize that these communities can also communicate among each other and coordinate themselves. Bacterial communities are known to "produce a number of small molecules that serve as quorum sensors, signals that bacteria use to determine the density of the local bacterial community and how many of their own species are present. The bacteria integrate these signals to cue group behaviors like biofilm formation or toxin secretion" [57]. Biofilms are an important aspect of the microbial communities in water pipes [58]. In both animal and plants, bacteria are generally found on many surfaces where nutrients are present, even in very minute amounts. Biofilms are formed "on surfaces in large part because nutrients are found in higher concentrations than in the open liquid (planktonic) area." Attachment to the surfaces is "facilitated by the microbial excretion of an exopolysaccharide matrix sometimes referred to as a glycocalyx" or EPS. Once established, "microcolonies in this microenvironment" form a protective structure or bacterial matrix, which "allow water channels to form between and around" themselves. This "has been likened to a primitive circulatory system where nutrients are brought in and toxic by-products are carried out" [59]. Microbial communities in each and every specific habitat they live in display very marked quantitative and qualitative differences and dynamic equilibriums. For instance, "many marine surface waters maintain steady populations of approximately 10^6 bacteria and 10^7 viruses/mL" [56]. In contrast, the microbial communities typically found in most water distribution's systems and water pipes, even in the cases of "perfectly safe water," generally contain approximately 10^2 bacteria/mL. Although a concentration of 100–300 bacterial cells/mL may seem high or low depending on expectations, it still amounts to "millions of nonpathogenic microbes in every glassful" of water. These numbers of bacteria in drinking water are not exclusive to tap water but they are also rather typical in commercial bottled water [58].

With plants usually one finds extensive bacterial communities in the root area, also known as rhizosphere. In the rhizosphere, setting aside the fact that "plant-microbe interactions are not limited to bacteria" but also involve an "intimate, long-standing relationships with viruses and fungi as well," generally "bacteria are fantastically abundant; there are up to 10^{10} bacterial cells/g of soil in and around plant roots. Bacteria are also tremendously genetically diverse—that same gram of soil may contain up to 10,000 different species of bacteria" [57]. Generally, "when bacteria form a biofilm around the roots of a plant, microbial pathogens and soil-dwelling parasites cannot gain access" because the niche is occupied. Moreover, it seems most "likely that many plant-microbe partnerships have evolved on this basis: the plant supplies carbohydrates to certain microbes" and in return "microbes produce a wide array of compounds that inhibit or kill competing microbes." In fact, it has been estimated "that up to 30 %" of the carbon which is converted by plants during photosynthesis "actually leaves the plant as exudate into the soil." It should be noted, however, that bacterial-plant interactions are not limited to the root areas, because above the ground also, in the region known as the phyllosphere, epiphytic and endophytic microbes can also "occupy spaces that otherwise might be an entry point for pathogens" and locally produce "compounds including toxins to deter grazers," volatile compounds that alert neighboring plants to the presence of a threat with small molecules that trigger protective responses of "closing of stomata" [57].

In the case of animals as well, many animal-microbe interactions involve communities, including bacteria, viruses, protozoa, fungi, and other microscopic eukaryotes. Animals, in addition to feeding upon the microbial biomasses, are also very tightly associated both to resident and transient microbes, as well as relying on very complex collaborations with bacterial communities in liquid and semisolid matrixes, biofilms, and symbiotic associations. For example, bacteria play "direct roles in the health of corals and other marine organisms." The "corals die when the bacteria that live on their surfaces are removed." "Bacteria associated with squid eggs have been shown to protect the eggs from fungal infection," and "biofilm bacteria" are "known to broadcast attraction cues that affect the settlement of invertebrate larvae in those biofilms" [56]. Moreover, both aquatic and terrestrial animals display many different strategies and specializations to access the nutrients in their habitats, where there are profound influences by resident and transient microbes. Filter-feeding marine bivalve animals, such as mollusks (e.g., clams, mussels, oysters, and scallops), which "feed by selectively filtering out small planktonic organisms, including bacteria, from seawater," in most situations, "carry a resident bacterial population that in the case of oysters fluctuates between 10^4 and 10^6 cfu/g of tissue, the higher counts occurring when water temperatures are high." However, when mollusks are constrained to live and "feed on polluted water, they concentrate contaminating bacteria, including enteric pathogens and viruses" [60]. This is most likely aggravated by the fact that they digest food through intercellular phagocytosis [61].

Large microbial communities in animals are usually associated with food digestion and with the consequent expulsion of waste products. For instance, jellyfish and sponges absorb food and expel waste from the same gastric pouches, while other animals process the food through a gut, which is characterized by a hollow tube beginning with a mouth and ending with the anus. The gut is comparable to an aerobic-anaerobic digester, where foods are sequentially broken down mechanically and then enzymatically in order to obtain simple sugars, amino acids, and small molecules, which can be absorbed and used by the animal [61]. All animal digestive systems involve at some level some assistance and collaboration from microorganisms, mostly by coordinated communities, composed of prokaryotes and/or prokaryote-eukaryote complexes. These communities not only protect the animals from pathogens but also actively take part in the process of digestion, as they actively break down “compounds and release nutrients that would otherwise be inaccessible” and synthesize vitamins and other molecules which benefit the animal’s health [62].

Complex polymers like lignin and cellulose cannot be degraded and used as a food source by termites and ruminants alike, without microbial consortia. According to Krause et al. [63], “rumen’s research has lead the way to microbial ecology,” at least since the earliest studies of Robert Hungate, who is rightly considered to be “the father of rumen microbiology.” Hungate first began studying termites and later moved to the study of the food metabolism by mammalian herbivores. Herbivorous degradation of cellulose is the result of complex “host-microbiome relationships” that can only be briefly mentioned here. Generally, facultative anaerobic bacteria begin by metabolizing liquids and soft tissues thereby reducing the oxygen levels in the biomass and creating a novel “anaerobic ecosystem.” Concurrently, “fibrous plant fragments” are colonized by oxygen-tolerant, “anaerobic zoosporic fungi” to continue the process. Interestingly, fungi are generally regarded as strict aerobes, but nature has rules and many “exceptions.” Their discovery is mainly credited to Robert Hungate’s innovative anaerobic culturing methods of rumen fluids and microbes. In fact, as facultative anaerobic bacteria begin by establishing new anaerobic conditions, this condition allows “anaerobic zoosporic fungi” to express their enzymatic “fibrolytic activity” in degrading fibers. Their activity softens the fibers and also “facilitate bacterial access to plant biomass,” which in turn favors “bacterial colonization and degradation” by cellulolytic microbes and strict anaerobes which further degrade the biomass. The process is progressive and resembles a “continuous culture system,” where the starting raw materials are processed in a “fermentation chamber, and regulates its further passage,” by “continuously absorbing the fermentation products, and transforming them into a few valuable substances such as meat and milk” [63].

Robert Hungate, during the 1940s and 1950s, “developed “roll tubes” to obtain thin layers of agar in test tubes, in which cellulose digestion and colony morphology could more easily be seen.” His successful attempts to grow “cellulose-digesting bacteria from the rumen of cattle” in the lab were based upon prior experimental attempts by other workers. Success was especially found in the presence of several determining factors: strict anaerobic conditions with an atmosphere saturated with carbon dioxide (CO_2), obtained by adding “bicarbonate to simulate the natural rumen habitat” to the media and also “rumen fluids,” which are “essential nutritional supplements,” as “the salivary buffering system” can be considered to be “central to the isolation of cellulolytic bacteria.” Later, in 1965, Scott and Dehority also demonstrated that “production of B vitamins by ruminal bacteria” is also “important to ensure bacterial growth and, particularly, fiber degradation” which is in turn “responsible for ensuring animal health and well-being” [63].

Another important scientific contribution that comes from herbivorous microbiology is the discovery of “syntrophic hydrogen gas (H_2) transfer.” Although “the first isolation of an organism that “oxidized” H_2 and reduced CO_2 was reported in 1933 by Stephenson and Stickland,” “the development of the Hungate technique was the major impetus for the isolation and characterization of methanogens from the rumen and other habitats." This mechanism of microbial interspecies transfer of hydrogen gas "was first hypothesized by Hungate in 1966" and can be described as a process where, during the degradation of organic materials during fermentation, hydrogen gas is produced by some microbes but is utilized by other species. This aspect has profound implications on microbial ecology and on the fermentative process because on one side, hydrogen gas favors the "growth of methanogens and benefits the fermentative bacteria, protozoa, and fungi," but on the other, an "accumulation of hydrogen gas" can also "slow down the fermentation rate and efficiency." So by this interspecies microbial collaboration both in natural ecosystems and anaerobic digesters, methanogens act as "hydrogen gas sink" and "reduce carbon dioxide (CO_2) with hydrogen gas (H_2) to produce methane gas (CH_4)" [63].

Anaerobic fermenters have been used for hundreds of years to produce methane gas and convert biomasses into renewable energy. "Current commercial-scale methods of methane production yield from 50 to 100 % conversion of substrate to methane on a mass basis, depending on the feedstock." However, this natural "end product of the anaerobic food chain" [64], especially in domesticated cattle and other ruminants, has fueled "current discussions surrounding global climate change" and the importance and roles of zoo technology "in regard to methane production and nitrogen runoff." In an attempt to curve methane production by the cattle rumen's microbiota, Wright et al. reported in 2004 the development of "a vaccine against specific members of the archaeal genus *Methanobrevibacter*". However, studies with this vaccine by different authors have indicated that the "change in the composition of archaeal populations after vaccination was accompanied by a significant increase in the diversity of the methanogen populations." Nevertheless, rumen research should also be credited for the discovery of "hyperammonia-producing bacteria from the rumen and other environments," but "their impact and role in the rumen is still being fully elucidated." Future research can potentially address also the problem caused by urinary nitrogen, which "represents an economic cost to the producer and carries an environmental impact that is becoming increasingly crucial to the animal industry." In any case, however, "rumen fermentations will likely always continue to produce at least some methane," and urine will also most likely continue to contain some nitrogen [63].

"Microbial communities have been found to adhere to the same basic rules of ecology that macro-communities follow," but in addition when compared to larger organisms, they generally possess more abilities to rapidly "engage in horizontal genetic exchange within communities" and are also more equipped with much greater "genetic diversity" and "metabolic plasticity." Microbial communities are "capable of recovering" from and rapidly adapting to "radical habitat alterations by altering community physiology and composition" and share the ability to maintain homeostatic stability; however, "when exposed to conditions that exceed this adaptive tolerance, a microbial community will destabilize." "These conditions, which can include nutrient starvation, viral infection, and other environmental insults, can be viewed as catastrophes for the community." Human activities, in particular, are "responsible for destabilization of communities." For instance, "supplying a diet of grain to cattle has been shown to lead to a lower rumen pH, which alters the gut community and fosters the growth of pathogenic strains like *Escherichia coli* 0157:H7" [65]. Globally, food processing by animals can be compared to a giant incubator, where most culturable and many nonculturable beneficial microorganisms dwell and reproduce. This has many local and planetary consequences in the planet's food web. However, sometimes, but not without some reasons, this giant and very efficient incubator can harbor among millions of "good bugs," at times, a few hundred species of "bad bugs" which are, of course, the zoonotic and the water and foodborne bacterial pathogens we all dread [66].

Chapter 6
The Fungi and Other Eukaryotic Microbes

Although the macroscopic fruiting bodies of fungi have been known from ancient history, and yeast fermentation has been practiced for thousands of years [18, 25], but "starting from the original concept of Linnaeus who makes a distinction upon two great kingdoms—the one of the Animals and the one of Plants—fungi have been traditionally placed among vegetables and were therefore the object of study of botanists" [67]. Taxonomical "binomial names in the classification of plants" were first used by Gaspard Bauhin in 1623, and his concept was refined, expanded, and consolidated by Carolus Linnaeus, in his fundamental treatises: *Systema Naturae* (1735), *Genera Plantarum* (1737), and *Species Plantarum* (1753). In 1749, Linnaeus revises *Genera Plantarum* and also expands "his system of binomial nomenclature and classification of plants by establishing not only species and genera but also classes and orders." He also farsightedly states that "each species has a specific place in nature, in geographic location, and in the food chain." In *Species Plantarum*, "Linnaeus gives a genus and species name to the 5900 plants he has classified." Remarkably, however, Linnaeus had described some "18,000 plant species" in his earlier work, but unfortunately "his earlier efforts had resulted in long Latinate descriptive terms tedious to use and difficult to remember." Although Linnaeus' scientific contributions on microbiology are not as convincing as the ones in botany, it may also be in part because Linnaeus, surprisingly, generally "doubted the usefulness of microscopic observations" and was also skeptical about the "existence of spermatozoa" [24].

In 1857, Carl Wilhelm von Nägeli "places the bacteria in the group Schizomycetes, the fission fungi, in the plant kingdom," and in 1866, Ernst Heinrich Haeckel first "suggests a system classifying living organisms into three kingdoms: Plantae, Animalia, and Protista." "He places the single-celled forms, the bacteria and some protozoa, into a group that he calls Monera, because he believes them to lack a nucleus." "Haeckel's ideas continue to influence classification of the bacteria and other single-celled organisms until the 1950s," when Herbert F. Copeland in 1956 first "suggests four kingdoms: Monera, the procaryotic bacteria and blue-green

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algae; Protoctista (or Protista), the lower eucaryotes such as algae, protozoa, slime molds, and fungi; the Plantae, photosynthetic organisms ranging from bryophytes to higher plants; and Animalia, the multicellular animals." The four kingdom classification is however replaced in 1969, when Robert H. Whittaker successfully "proposes a five-kingdom system of classification of living organisms: Plantae, Animalia, Fungi, Protista (protozoans and algae), and Monera[1] (blue-green algae and bacteria)," which rapidly becomes "widely accepted" [24].

There are therefore many possible definitions of fungi. Ronald M. Atlas (1988) defines fungi as "a group of diverse, widespread unicellular and multicellular eukaryotic organisms, lacking chlorophyll and usually bearing spores and often filaments" [68]. While Gherbawy and Voigt (2010) emphasize different aspects of the unique underlying characteristics of this kingdom: "fungi are originally heterotrophic eukaryotic microorganisms harboring chitin in their cell walls and lacking plastids in their cytoplasm" [69]. It is interesting to notice that many scholars have considered fungi to be closer to animals than plants. In fact, chitin is a "polymer, which in microbes is only found in fungi" [63], but is very common among arthropods and may be present also in other invertebrates. "Recent studies have revealed that fungi are more closely related to animals than many other eukaryotic organisms" as probably these two "kingdoms diverged from their last common ancestor (a unicellular organism that lived in the oceans propelled by a flagellum) on the order of a billion years ago." "Fungi are eukaryotic, heterotrophic organisms" and "come in three basic shapes: unicellular yeasts, filamentous hyphae (molds), and, among the most basal groups, flagellated, swimming, unicellular organisms that encyst to form sporangia." "The yeasts, hyphae, and sporangia have cell walls that contain some of the rigid polysaccharide chitin, along with a variety of glucans" [70].

Fungi can be present in the air, in fresh and salty water, in the soil, and in a multitude of Earth's environments. They can usually reproduce sexually and asexually. They generally digest food through extracellular digestion and behave as saprophytic, symbiotic, or pathogenic organisms [67]. As saprophytes, they are known to be "primary degraders of organic matter" and are therefore "responsible for turning dead plants into small nutrient blocks other organisms can use." Entire "ecosystems rely on fungi to degrade organic matter and mineralize the products, making essential elements available for uptake by plants and bacteria." It has been estimated that "in tropical rain forests, ~50 % of the dead plants and animal matter (by weight) is degraded by fungi." Also "many crops, forests, and other ecosystems rely on interactions with fungi to provide nitrogen and other nutrients. Roughly 60 % of plants interact with fungi in ways that benefit the plant." As symbiotic organisms, "fungi maintain a myriad of different symbiotic relationships with animals, plants, insects, amoeba, bacteria, and other fungi." Particularly in regard to plants, mycorrhizal fungi are known to interact with "almost all vascular plants," and some of them, "such as orchid species, are totally dependent on their fungal partner to germinate and grow" [70].

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[1] The Monera are usually and generally now called "Procaryotae" or prokaryotes.

Fungi are not only indispensable in the rumen metabolism but are also necessary in many more vertebrate and invertebrate organisms. Scientifically their discovery as essential "anaerobic component" of the ruminal microbiome has "disproved the central dogma in microbiology that all fungi are aerobic organisms" [63]. As pathogens, fungi are also formidable organisms because they not only can incite "disease in humans, plants, animals and insects," but certain fungi can also act as "trans-kingdom pathogens" where the same species is able to "infect both plants and animals, overcoming significant differences between these very different hosts" [70].

Fungi also produce a very vast number of different enzymes and secondary metabolites. In general, "secondary metabolites are compounds that are not necessary for growth and reproduction" and "fungi make them for a variety of reasons, including self-protection from predators, such as mites and amoeba, killing competing fungi and bacteria, and signaling to nearby microbes" [70]. Although some fungal secondary metabolites are also very useful to human medicine, others are very toxic to humans and to other animals. Humans have been using "fungal secondary metabolites as life-saving medications," at least since 1929, when "Alexander Fleming discovers an antibacterial substance in a culture of the mold *Penicillium notatum* and "names the substance penicillin" [24]. For thousands of years, "many fungi and fungal enzymes" have been successfully "used in industry in processes ranging from fermentation to food production to the conversion of biomass to ethanol." In the "biotech industry," "yeasts and other fungi are presently being used as production machines for vaccines, vitamins, monoclonal antibodies for use in immune therapy, and other therapeutics." In addition, besides "antibiotics," fungi also synthesize other important compounds for human medicine, "such as cyclosporin A, a revolutionary drug that inhibits the rejection of transplanted organs, and statins, which are widely used to treat elevated lipid and cholesterol levels and reduce the risk of heart disease." Traditionally, however, food production has been most likely the first human activity to benefit directly from the metabolism and the versatility of fungi. "All leavened bread, all alcoholic beverages, vinegars, citric acid-based beverages, the Roquefort and Camembert cheese families, and many Asian foods, such as tempeh, soy sauce, and miso, are among the many foods produced with the assistance of fungi." Furthermore, all microscopic and macroscopic fungi serve in their turn as food sources and are "consumed by many organisms, including animals, insects, worms, bacteria, and other fungi" [70].

Currently, "yeasts may be viewed as being unicellular fungi in contrast to the molds, which are multicellular; however, this is not a precise definition, as many of what are commonly regarded as yeasts actually produce mycelia to varying degrees." "Yeasts can grow over wide ranges of acid pH and in up to 18 % ethanol. Many grow in the presence of 55–60 % sucrose" and can therefore survive and grow on most food substrates [71]. It is generally known that "yeasts are exploited in the production of alcoholic beverages, notably beer and wine" as well as in "baker's yeast for bread dough production" [72]. However, notwithstanding that yeasts "are particularly resistant to dry conditions when compared to bacteria" and can "also tolerate acids," they do not only represent a source of contamination in other foods, but they are also present and actively inoculated along with other microorganisms.

Many dairy products in different parts of the world such as "Kefir," "Koumiss," and "Skyr," are all fermented with yeasts and contain "small amounts of alcohol" [73]. Yeasts, however, are not only important in "fermented milks and creams," but they are also important in cheeses, where they are not just a source of contaminations and spoilage. In fact, in some traditional cheeses, yeasts are a contributing part to the "natural contamination" of "surface-ripened cheeses" and take part in the "development of a multitude of flavor sensations in cheese as it aged." "Yeast and molds are common on surfaces of "rind cheeses," a large group of traditional European cheeses." These cheeses are usually not covered or packaged in a conventional way, but rather, they are generally allowed to mature "in air," since "growth of mold and yeasts is expected if not demanded," by traditional consumers [74].

Many well-known types of cheese are also actively or naturally inoculated with different molds and with other microorganisms because they "produce flavors and cause desirable changes in texture and appearance of the finished cheese." The fungal inoculum can either be directed to the surface of the cheese or can also be targeted to the interior. In the case of "Roquefort, Gorgonzola, and other blue cheese types," "although spores of *Penicillium roqueforti* are added to milk or curds before the lactic fermentation, mold growth does not occur until the lactic culture has fermented all or most of the available lactose to lactic acid." The reason for this is that "lactic acid serves as an energy source for the mold." In turn, "consumption of lactic acid" by the mold causes the pH of the substrate to increase, which is accompanied by substantial proteolysis and lipolysis from the molds. Once proteolysis begins, amino acids are further processed to release "amines, ammonia, and other possible flavor compounds" which also increase the pH. "The most characteristic blue cheese flavors" are however "generated from lipid metabolism." It was found that during lipolysis, "as much as 20 % of triglycerides in milk are hydrolyzed" into "free volatile fatty acids," which in turn through "ß-oxidation pathways result in the formation of a variety of methylketones," which are the molecules responsible for the typical and characteristic "flavor of blue cheese" [75].

In the case of Camembert and Brie cheeses, the inoculum provided is instead the fungus *Penicillium camemberti*, which also interacts in similar ways with lactic acid bacteria and to changes in pH. This fungus, however, only grows on the surface of these type of cheeses, mainly because unlike blue cheeses, "no deliberate aeration" of the cheese mass is provided "during cheese making," which is designed to allow the fungus "to grow throughout the cheese mass." In this instance, the fungal action throughout the cheese mass does not depend on the direct colonization but depends on the diffusion inside the mass of excreted enzymes. The fungal activities result in the "production of ammonia, methanethiol, and other sulfur compounds presumably derived from amino acids" which, along with "lipolysis of triglycerides and fatty acid metabolism" and consequent production of "methylketones," which account for the organoleptic "characteristic of Camembert cheese" [75].

Fungi are important in a wide variety of different foods including "dry-cured salami" and other meat products such as "country-cured hams" and similar types. In general, "the curing and ripening period," which lasts months and up to even 2 years or more, according to the relative moisture and storage conditions, results in "heavy mold growth," on external surfaces. In these particular instances, Jay et al. remind us that Ayres et al. in 1967, "found nine species of penicillia and seven species of aspergilli on fermented sausages and concluded that the organisms play a role in the preservation of products of this type". And Andersen in 1995, while studying "the fungal biota of naturally fermented sausages in northern Italy" and "revealed that penicillia made up 96 % and aspergilli 4 %." He also observed the evolution of the fungi and found that "the initial biota of the sausage was made up of >95 % yeasts. After 2 weeks, yeasts and molds were about 50:50, but after 4–8 weeks, molds constituted >95 % of the biota." Ayres et al. (1967), in "country-cured hams" which are made in the southern USA, "noted that the presence of molds is incidental and that a satisfactory cure does not depend on their presence"; however, he also indicated that "it seems likely that some aspects of flavor development of these products derive from the heavy growth of such organisms and to a lesser extent from yeasts." He also "found aspergilli and penicillia to be the predominant types of molds" in this product also. Generally, "heavy mold growth" can hinder "the activities of food-poisoning and food-spoilage bacteria" on the surface of the products, "and in this sense, the mold biota aids in preservation." "In Europe, molds are critical in the production of safe and high quality products such as salami and hams" [76].

Fungi interact and also compete with the human food supply in many different ways. Depending on conditions and context, fungi can either be friends or foes, as they are able to provide and improve our foods and drinks, they can also be a source of contamination and spoil them. Yeasts in nature are not relatively "tamed" like the *Saccharomyces* we know in homemade and industrial fermentations, as "there are many different genera and species present," for instance, during the process of "vinification that ultimately impact quality, both positively and negatively" [77]. The same is true for many fungi in food, which can at times be an essential source of enzymes and flavors or a cause of spoilage and contamination, on occasions just unsightly, but also frequently through their invisible synthesis of toxic compounds which renders the foods inedible [78]. Fungi are also in some occasion very aggressive parasites and "indoor environmental pollutants," but they are not just a microbial community of parasites, for there are many specialized parasites, in each and every one of the five kingdoms. Numerically, among "roughly 1.5 million fungal species estimated to be alive today, only some 200 have been associated with the human body so far, either as pathogens or as commensals, and of these, a dozen or so represent the most common fungal pathogens" [48].

It must be clearly pointed out that there are many more microbial eukaryotes than fungi. In particular, the kingdom "*Protista*" should not be forgotten or underestimated because it displays numbers and variability probably as important and complex as the remaining four kingdoms. All the five kingdoms are also interacting in ways we are just beginning to understand. Fungi are certainly not the only eukaryotic microbes which can produce toxic secondary metabolites; some algae, for instance, are cultivated for food and energy, while others are toxic and transfer their toxicity through different aquatic food webs to fish and shellfish, thereby posing a serious threat to humans because these toxins are not inactivated by heat [78]. Saprophytic and predatory "free-living protozoa" are also "ubiquitous in natural ecosystems where they play an important role in the reduction of bacterial biomass and the regeneration of nutrients" but cannot be dismissed or just "considered as predators of bacteria." In fact, their "role in survival and protection" of bacteria, including food pathogens, is "increasingly being recognized." In respect to the interactions of "free-living protozoa and bacterial food pathogens" to date, there appears to be still "some conflicting results," in terms of the "survival and/or replication" of this prokaryotes and the "intracellular viability of bacteria in protozoans" [79].

In conclusion, as Linnaeus as early as 1749 had already pointed out, "each species has a specific place in nature, in geographic location, and in the food chain." Clearly this concept must include all parasites, opportunists, predators, toxic and poisonous animals and plants, as well as microorganisms [24].

Chapter 7
The Human Behavior and Food Resources

According to taxonomical conventions, we have classified and designated ourselves as *Homo sapiens*. "Sapiens" is a Latin word similar to the Italian word *Sapiente*, which is analogous to English *Sapient* (adjective, *sagacious*), for which some synonyms are available such as "acute, astute, clever, clear-sighted, intelligent, judicious, insightful, informed, experienced, discerning, perceptive, reflective, sensible, educated, thoughtful, knowing, smart, knowledgeable, scholarly, discriminating, contemplative, enlightened, far-sighted, sage, wise, sharp," and also some rather less "flattering synonyms," such as "shrewd, astucious, and canny" [80]. In taxonomy, generally genus and species are not sufficient to adequately describe the diversity within each species. Therefore, our precise and unique taxonomic classification is *Homo sapiens*, subspecies "sapiens," also generally known as "anatomically modern humans." According to anthropology and paleontology, available records suggest "the earliest evidence of anatomically modern humans comes from around 30,000 to 35,000 years ago." Additionally, since we have been very mobile primates from the beginning of time, it is generally accepted that around the era known as "the Upper Paleolithic," "the hominids expanded to their current distribution" [81, 82]. Presently, according to the estimates of the "United States and World population clock," which is available and constantly updated on the "United States Census Bureau" website, the estimated "World Human Population" is believed to be "7.3×10^9 individuals" [83]. Considering that the number of individuals of a population is generally limited by the availability of resources, from the beginning of the "gathering and hunting societies of the Paleolithic" to the "agricultural communities of the Neolithic" to modern times, hominids have traveled extensively in search of new food sources, whereby also developing new strategies and innovative methods to obtain and to handle foods [15].

In this respect, evidence seems to indicate that during the "Upper Paleolithic," Americas were settled by "human groups" who had apparently first colonized the area presently known as Siberia and then involuntarily "migrated across the Bearing Strait into North America and soon after into South America." It is also hypothesized

A. Zaccheo et al., *Food Hygiene and Applied Food Microbiology in an Anthropological Cross Cultural Perspective*, DOI 10.1007/978-3-319-44975-3_7

that this passage “was not by a purposeful migration but probably by human groups following herds of large game animals.” More or less concurrently, Australia was also “settled by human beings from the Asian mainland” [82]. According to the Cambridge World History Of Food (excerpt), in the Andean regions of South America, Ugent in 1970 proposed that potato tubers have been consumed and perhaps also cultivated by natives as early as “7000 years ago” [84]. Many years later, upon the rediscovery of the Americas by the Europeans, the potato was introduced “into Europe about 1570.” Subsequently, “in Ireland between 1750 and 1840, the increased food supply resulted in almost a tripling of a population, a population dependent almost solely upon the potato.” But “around 1830 the fungus which causes potato late blight”, *Phytophtora infestans*, “hitched a ride” to Europe, probably carried on soil and plant material, and arrived in Ireland around 1843. There, “the cool, humid climate of Ireland was ideal for the fungus which quickly annihilated the potato crop.” “Because the potato crop was grown primarily to feed the Irish peasants,” whereas other crops “had to be paid to the landowner for rent,” the vast majority of the Irish population was suddenly without one of their main staples and food source. It has been estimated that “about one million people died of starvation or disease brought on by malnutrition” and “another million or so emigrated, mainly to the United States or Canada” [40].

In the case of Old World grapes *Vitis vinifera*, human migrations also had an enormous impact. The plant is generally “thought to have originated in Asia Minor” and have “been cultivated for some 6000 years.” Grapes were probably first cultivated by the Egyptians and then later were brought to Europe first by the Greeks and then by the Romans [18]. Since the importance of the grape as a food source is more marginal than a primary staple such as potato, fungal grape diseases resulted in far less dramatic conditions and situations than the ones just described in Ireland. However, “through the 1800s France was one of the largest producers of wine in the world.” The French winemakers were therefore entreating excellent business relations with the once extensive “British colonial empire,” and “England had become a major importer of wines and brandies” destined mainly to the upper economical classes. When the fungal diseases “powdery mildew” and “downy mildew” later arrived in Europe from North America from 1840 to 1878, the diseases rapidly spread throughout the continent and soon became “established in virtually every vineyard in France.” Even when French botanist and mycologist Alexis Millardet “discovered Bordeaux mixture” in 1884, “the first commercial fungicide,” still widely used today, “it was too late.” “By the time that the vineyards had fully recovered from the disease,” the clients of the English “upper classes” had switched from “brandy” “to the most available item on the market”: whiskey, which is still largely true today [40].

Different sets of situations are associated with the coffee plant *Coffea arabica* instead. The plant “probably originated in Ethiopia” and “was taken from there to the Arabian Peninsula by Arabic traders perhaps as early as 500 AD” to be cultivated. Arabs “prized coffee, as they do to this day.” Only small amounts of coffee was initially “exported to Europe around the 1500s” as “Arabs jealously guarded the source of the beans and let no one obtain seeds for planting.” Apparently in the

1600s, some Indian Moslems managed to smuggle "a few seeds back to India," where some "Dutch traders" eventually found those plants and "took some of the younger plants to Java and Sumatra" to be cultivated. Coffee became very popular especially in England "during the 1650s," as historians recorded that thousands of "coffee houses" existed in London alone at the time. Coffee houses in that period "were the favorite meeting place of politicians, writers, and socialites of all ranks." Fearing possible risks of "political intrigues hatched" there, "King Charles II banned all coffee houses in 1675," but, apparently, "the hue and cry were so great that he was forced to rescind his order 10 days later." In the following years of the 1700s and 1800s, the British colonial empire "encouraged the founding of huge coffee plantations" overseas in the island of Ceylon. The island presently known as Sri Lanka became one of the largest coffee producers of the world, as "by 1870 Ceylon was exporting 50 million tons of coffee per year." "But around 1870" their plantations became progressively infected by the coffee rust fungus *Hemileia vastatrix*, and "by 1892 the plantations had been completely wiped out, and Ceylon was importing coffee for his own use." This disease had great economic implications, as "plantation owners went bankrupt; some committed suicide," and the "Oriental Bank failed." "But some of the planters hung on" and rapidly switched to what was considered at the time "a minor crop": tea. From "1875 and 1880," "three hundred thousand acres" of tea were planted to replace as much as "three-fourth of the former coffee plantations," "and the English became a nation of tea drinkers, which they are to this day." In the following years, "the center of coffee production shifted to South America, where the pathogen did not occur" [40].

Based on these illustrations, one might be tempted to conclude that pathogenic organisms tend to express their devastating effects mainly on imported or exotic crops cultivated as single crops or in "monoculture." However, this is only partly true as it is demonstrated in the case of "chestnut blight," the fungus formerly known as *Endothia parasitica*.[1] This fungus, in just 26 years, devastated the native wild American chestnut tree, from 1904 when it was first found "in the New York Zoological Park" to 1930 where the fungal disease had "virtually annihilated the American chestnut as a forest tree from Maine to Georgia." The pathogen, however, did not manage to completely "eradicate the American chestnut as a plant," because incidentally "the fungus does not kill the roots," and luckily "the chestnut happens to be a very prolific sprouter." As a result, even though "the American chestnut has been eradicated as a forest tree, it is still present throughout the Appalachians as an understory shrub." According to Merril [40], however, "this is the only example we know of where a pathogen has almost annihilated a wild plant species" [40].

In present days, humans have become increasingly aware and alert in regard to other invasive species and their detrimental effects to biodiversity as well as the environment. However, invasive animal or plant species along with their macro- and microorganisms can occur even more today, as we travel and trade more extensively and intensively than ever. Most foreign species are not invasive, but highly invasive

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[1] The sexual stage of the pathogenic fungus *Endothia parasitica* is now referred to as *Cryphonectria parasitica*.

species can rapidly relocate either through “natural pathways” and frequently “may involve some direct form of human mediation responsible for the movement of a species” or some “habitat disturbance,” which in turn facilitates the invasion of “aggressive” allochthonous species [85, 86]. In fact, no other animal species on Earth has changed so drastically and so extensively their own habitats, as well as the ones of other living organisms. According to Ron Hoggan in 2010, based on the publication of William R. Catton in 1980 and 2005, “current estimates suggest a world population of about 3 million at 35,000 B.C.” [87, 88], which through some “compound interest” calculations, results in an estimated “80 millions people by 4000 B.C.” [88]. From Neolithic times, the rural activities have been growing more or less steadily to keep pace with the food demand. Now, however, according to the UN Department of Economic and Social Affairs, “in 2007 for the first time in history, the global urban population exceeded the global rural population, and the world population has remained predominantly urban thereafter.” Moreover, “the rural population of the world has grown slowly since 1950 and is expected to reach its peak in a few years.” “However, sometime after 2020, the world’s rural population will start to decline and is expected to be 3.2 billion in 2050” [89]. In contrast, according to these same estimates, “the world’s urban population increased from 2.3 billion in 1994 to 3.9 billion in 2014 and is projected to grow to 6.3 billion by 2050” [90].

We must keep in mind, however, that these average “levels of urbanization vary greatly across regions” of the world, as “high levels of urbanization, at or above 80 %,” are found in “Latin America and the Caribbean and Northern America,” second to “Europe, with 73 % of its population living in urban areas,” where it “is expected to be over 80 % urban by 2050.” In contrast, “Africa and Asia” have remained “mostly rural, with 40 and 48 % of their respective populations living in urban areas.” But it should be also noted that “Africa and Asia are urbanizing more rapidly than other regions of the world,” with an average increase “by 1.5 % and 1.1 % per annum, respectively.” In general, “the urban population is expected to continue to grow, so that by 2050, the world will be one-third rural (34 %) and two-third urban (66 %) roughly the reverse of the global rural–urban population distribution of the mid-twentieth century” [89]. These changes in the living patterns are likely to cause, in addition to urban sprawl, profound changes in water use and in food logistics. Consequently these changes affect food security and food safety as well, because a greater share of different foods for increasingly larger urban population will most likely have to be transported for greater distances between the rural environments where production occurs, to the increasingly larger and more extensive urban environments where consumption takes place.

Probably also as a direct consequence of urbanization, the global fertility rate for the total human population has been steadily declining for several decades from now. In the last 40 years, the average human fertility rate has dropped “from around 4.5 children per woman in the early 1970s” “to around three children per woman in 1994” and is estimated to be approximately “2.5 children per woman in 2014.” This “decline had been particularly sharp in Asia and Latin America and the Caribbean, whereas fertility decline had just begun in Africa” at a slower pace.

A “notable exception” in this trend is Europe, which has “rebounded slightly in a number of countries,” although it is still considered to be insufficient “in most cases, to reach replacement level” between births and deaths. In addition, “life expectancy at birth for the world as a whole rose from 64.8 years in the period 1990–1995 to 70.0 years in the period 2010–2015, a gain of 5.2 years.” “Although the gap in life expectancy at birth between the countries in the more developed regions and the least developed countries remains large, at 17.1 years, it is nonetheless 5 years smaller than in the early 1990s.” The change in life expectancy ranges from “3.3 years in North America,” “6.5 years in Africa,” and remarkably “8.9 years in the least developed countries.” Furthermore, “considerable progress” has been also achieved in “reducing child mortality” in the last decades. Child mortality “is estimated to have fallen by 40 % between 1994 and 2014.” However, close to “one quarter of the countries in Asia are expected to be unable to achieve” the millennium goal of reducing “the under-5 mortality rate” by two-third by 2015. Furthermore, in many least developed countries in Africa, a child is estimated to be “14 times more likely to die before age 5,” compared to “North America or Europe” [90].

So while on one hand, the general increase of the “life expectancy is a success story,” and because in many parts of the world not only “people live longer,” but also “choose to have fewer children,” the reverse side of the medal is that population aging becomes more predominant, and older people are becoming “a proportionally larger share of the total population.” De facto, “older people are presently the world’s fastest-growing age group, and in 2014 the annual growth rate for the population aged 60 years or older” is going to “almost triple the growth rate for the population as a whole.” “In absolute terms, the number of people aged 60 years or older has almost doubled between 1994 and 2014, and people in this age group now outnumber children under the age of 5.” In view of these circumstances, it is not only the drastic change and impact of the “old-age support ratios” between the “working-age adults” and the older people but especially in less economically stable areas, and in places “with limited social security systems,” older people are also becoming at a “much greater risk for poverty” [90]. These situations can therefore be reasonably expected to increase and in turn, the number of individuals who are affected by water and foodborne diseases as well.

Chapter 8
The Human Microbiomes

It took indisputably thousands of years for humans to simply realize the mere existence of microbes. Even though we live in a microbial world and despite many defeats in our constant fights against the "bad bugs," we are just beginning to get a grasp at understanding some of the complex dynamics that govern interactions between microbes, environment, and humans. Historians in a foreseeable future may possibly describe our era as a revolutionary period, characterized by an enormous impetus to better understand the "humble microbes," which subtly govern every single aspect of our lives in many ways. Culture-independent molecular genetic methodologies are revolutionizing the way we look at microbes in their interactions with other organisms. Until recently, the living organisms found in complex communities and assemblages were generally described as the "microflora," "but this term is now considered incorrect and misleading, since it implies that humans are colonized with tiny plants." There are two possible definitions or visions regarding the modern term "microbiome," one that comes from the field of molecular genetics and the other that comes from the study of ecology. The molecular genetics definition of "microbiome" refers to "the entire collection of genes found in all of the microbes associated with a particular host." The "broader term," "metagenome," is "the entire collection of microbial genes found in a particular environment," which "may or may not be host associated," such as the communities and assemblages which can be observed in "sea water, showerheads, hot springs," bathtubs, or soil. In ecology the term "microbiome" is instead used "to describe the collection" of the many different living microorganisms "that live in a particular environment characterized by similar climatic conditions." Therefore, in the context of microbial ecology, the "human microbiome" can be viewed as the entire "collection of microbes that live in the human habitat" [91–93].

The human body comprises approximately "3.7×10^{13} human cells," but it also includes as many as "1.0×10^{14} bacterial cells" along with other microbes. The human microbiome to date is estimated to constitute a mass around 1.1 kg or "2.5 lb" and a volume of about 1.4 L or "3 pints." Different microbial communities

A. Zaccheo et al., *Food Hygiene and Applied Food Microbiology in an Anthropological Cross Cultural Perspective*, DOI 10.1007/978-3-319-44975-3_8

tightly associated with humans are found “wherever the human body is exposed to the outside world,” such as “the entire surface of our skin,” “the linings of our nasal passages,” the “lungs,” the “oral cavity,” the “digestive systems,” as well as the “urogenital tracts.” “At birth, we get our microbiome mostly from other humans,” first generally from the “mother’s birth canal” and/or from the “skin of its mother”; “from their fathers, siblings, and caregivers”; and most importantly in the next few weeks and months from “breast milk,” from which the newborn acquires “diverse microbes to populate the baby’s gut,” which are not only destined to simply coat and occupy the intestinal surfaces “and preventing the attachment of pathogens” but are also present to actively break down “complex carbohydrates (oligosaccharides) and glycosylated proteins” that “cannot be digested by the infant.” “Bifidobacteria are the dominant species in the infant microbiome,” where they play a dual role of “probiotics” or “beneficial microbes” as well as producers of “prebiotics,” whereby generating compounds that “support the growth and establishment of beneficial microbes.” Breast milk, moreover, in addition to “nutrients for the baby” and to the “baby microbes” supplies also the infant with essential “vitamins and antibodies” [91–93].

As we land into this world, and throughout our whole life, independently of where we are from birth and for the rest of our life, we will “continuously encounter” and interact with thousands of species of “new microbes,” coming from every conceivable places on Earth such as air, water, food, soil, animals, plants, and of course other people. At first sight from the viewpoint of a straight cell count, “a human body is actually only about 25 % human cells.” Howbeit molecular genetics provides an even clearer sense of magnitudes and proportions. If one compares, for instance, the number of microbial genes to human genes in the human microbiome, prokaryotes alone would account for and contribute to at least “an extra 2,000,000 genes to the 20,000 gene human genome.” This corresponds approximately to a “proportion of 1 % human genes to 99 % microbial genes.” This estimate, however, does not take into consideration that in human microbiomes, “viruses outnumber bacteria” by a factor of “5:1,” and fungi are generally “10 times fewer” than bacteria. Therefore, “the collective genomes of all the bacteria, fungi, and viruses in one person’s microbiome are thought to include as many as 8,000,000 genes.” As a rough estimate, it can be stated that “for every human gene, there are up to 300 non-human genes” [91–93].

Despite these enormous numbers and staggering proportions, another important aspect “to recognize is that these associations are not random,” as each and every “organism has evolved to have intimate associations with particular kinds of microbes.” We must also acknowledge that microbial selection in all microbiomes occurs as a continuous process, both between “established” and “newly encountered microbes” and also by the host determined information and specific cues. Interactions through “specific markers” and complex “chemical communication,” and by means of “physical cues like temperature and moisture levels” and also through other extrinsic and intrinsic factors, determine where microbes can possibly grow and/or prosper. As a consequence of these complex interactions between microbes and the host, microbial associations are therefore not uniformly distributed on the human body, but, instead, each specific location which directly or indirectly interfaces with the environment will also harbor different and highly specialized communities within microhabitats, each having very distinct roles, precise functions, as well as specific densities. For instance, generally the "blood and lymphatic fluids are practically sterile," while by comparison "the intestines and colon contain one of the densest known microbial communities on Earth." Likewise, the microbiomes found on human skin, according to their relative position, moisture level, and presence of oils, are also known to greatly vary both in microbial compositions and numbers. Furthermore, "there are indications that some commonly found skin microbes can help keep away pathogens. For example, the frequently found Staphylococcus epidermidis has been shown to produce compounds that inhibit the related, but pathogenic, Staphylococcus aureus" [91–93].

Also in the case of oral microbiology, oral microbiologists have long recognized that oral microorganisms live on different types of biofilms "as compositionally and structurally complex mixtures of species" which are adhering to specific oral surfaces, such as is the case of the "archetypical example" of dental plaque, for instance. Not only "oral biofilms appear to have a highly ordered structure" but they are also organized on very "specific associations among different species of bacteria," where each community is specific not only to each other but also to the host's distinct mineralized and soft tissues surfaces. In the numerous oral biofilms where different bacterial communities adhere, they cooperate for food, interact among each other, and antagonize other microorganisms. For instance, in the "consumption by the host of a diet that is rich" in simple sugars, this in turn "leads to bacterial production of organic acids, such as lactic and acetic acids," which can bring "the pH of oral biofilms down to values of 4 and below." Eventually and "over time, the acid-sensitive organisms can no longer compete effectively, leading to the enrichment in cariogenic biofilms of organisms that are acid tolerant." Concurrently, some oral streptococci can also produce in biofilms a common disinfectant: hydrogen peroxide (H_2O_2), which "is toxic to a number of bacteria that lack, or have low levels of, the enzymes needed to detoxify oxygen radicals," thereby "making the H_2O_2-producing organisms more competitive." Moreover, oral biofilms are also known to produce some "peptide antibiotics" known as "bacteriocins." For example, the case of *Streptococcus mutans*, "through production of bacteriocins," "is able to suppress the growth of other streptococci that would otherwise compete for similar nutrients." The oral biofilm communities "are an important contributor to tissue homeostasis in the oral cavity." However, these communities can also be easily destabilized by many factors such as an increased amount of "carbohydrates" and especially of simple sugars "in the diet" or a "reduction of salivary flow," which both "lead to a sustained lowering of the pH of the plaque." Therefore, a current and more frequent approach "to maintain oral health" relies on "the concept of stabilizing a microbiota that is compatible with health" [94].

Furthermore, the importance in the very complex "oral environments" of saliva, mucus, and other substances, which are produced by the host in the "oral ecology" to protect the host's tissues and sustain beneficial microbial communities, can only be briefly mentioned here. We should however retain at least that specific microbial communities on teeth, tongue, gums, and other tissues are either associated to oral health or oral diseases. For instance, "the epithelium covering the gingival, buccal, and palatal tissues is also a habitat for microbes." However, "because the surface cells of epithelia desquamate at a regular rate (thus shedding attached bacteria), the soft tissues do not support the voluminous microbiota seen on the surface of the teeth." Nevertheless, as cited by Lamont et al., studies by Rudney et al. in 2001, "suggests that human buccal epithelial cells contain intracellular bacteria, including the periodontal pathogens," "as well as other species of bacteria." This situation "may allow bacteria to establish themselves in the mouth and in a habitat protected from host defense factors such as salivary antimicrobial systems." Saliva also plays many important roles for the host organism such as "in food digestion by solubilizing food components to facilitate taste" and also "by preparing the food bolus for swallowing" and, of course, "by providing enzymes to initiate digestion." Moreover, "the adsorbed salivary molecules also lubricate tissues to facilitate chewing, speaking, and swallowing and prevent the oral tissues from drying." Naturally, saliva also plays many roles in host microbial interaction in regard to the nutrition of bacteria in biofilms, by "buffering acids" and neutralizing "toxic products produced by oral microorganisms." In saliva, there are also many different antimicrobial compounds including "agglutinins, lysozyme, histatins, peroxidase and thiocyanate, and lactoferrin" [95].

Animal-microbial and human-microbial digestive communities are most likely not just species specific but can also vary considerably with the geographical locations and with their cultural diet or even just by what people usually eat and drink. Since the bacterial genome is so diverse and displays a great amount of plasticity and ability to horizontally transfer man genetic traits, "bacterial genomes can change dramatically more quickly than the human genome." This mechanism therefore provides "much more rapid means for humans to adapt and thrive when environmental conditions change." One interesting example of such adaptations "is the discovery of a gene for digesting seaweed in the microbiome of some Japanese people." This particular gene is "rarely found in human microbiomes outside of Japan," but it is abundant as "environmental bacteria that feed on seaweed in nature." "Possibly at some point, one such environmental bacterium," conceivably "while passing through someone's gut on a piece of seaweed, transferred some of its genes to a normal bacterial constituent of the human microbiome," thereby improving the genetic metabolic abilities of the gut's microbiome, by conferring also "the ability to digest seaweed," which is a "common part of the Japanese diet." In general, it is probably reasonable to assert that such mechanisms are much more common than what we would have predicted before the advent of microbial "culture independent molecular genetic methodologies" [91].

In all humans, we must remember that "the inner surfaces of the human intestine and colon are highly convoluted" to such extent that if one theoretically were to "flatten out the entire inner surface of the intestine" and measure the corresponding surface, it would easily amount to "the size of a tennis court." Historically, "the gut is the best-studied site in the human microbiome," where it has been shown to harbor "the largest, densest, and most diverse microbial community in the human body." As an order of magnitude, "the microbial community in the human large intestine" can be estimated to be from a lower bound of "100 billion–1 trillion cells per mL," which is likely one of "the densest microbial ecosystems ever observed" [91]. In regard to gastrointestinal microbes, most people have heard at least once about *E. coli* or *Escherichia coli*. The bacterium was first discovered in 1885 by "German pediatrician Theodore Escherich" [62], who isolated from "the feces of infants" two bacterial species named then *Bacterium coli commune* and *Bacterium lactis aerogenes*, since the latter microbe caused "the clotting of milk more actively than the former." During his research, Theodore Escherich "also finds that some strains of *Bacterium coli* cause diarrhea in infants." Following the usual changes in taxonomic conventions, "in 1919, Aldo Castellani and Albert Chambers change the name of *Bacterium coli* to *Escherichia coli*," while the former *Bacterium aerogenes* is currently named *Enterobacter aerogenes* [24].

Although "*E. coli* is a minority member of the gut microbiome," it is a very important one, not only for its role as a facultative anaerobe which first "consume oxygen from the gut," thereby establishing "a welcoming habitat for the other microorganisms of our microbiome," but also to the advancement of science. In this regard, *E. coli* was the first organism selected by geneticists to have its complete "genome sequenced" and many fundamental discoveries that "have conferred the greatest benefit on mankind," and among others, at least 11 Nobel Prizes have been awarded to researchers from 1958 to 2008 because of the involuntary contribution of this common bacterial organism [62]. Notwithstanding that the human gastrointestinal microbiome is composed of many commensal microbial species, "the composition and activity of this complex microbial system" are known to "have a major influence on health and disease." They are known to "contribute to the trophic functions of the gut (producing fermentation products and vitamins that can be used by intestinal epithelial cells)." They also "stimulate the immune function of the gastrointestinal tract, transform or excrete toxic substances, protect the host against invasion by pathogenic species, and modulate gut motility." Furthermore, Mai & Morris in 2004, indicated that "research incriminates a dysfunctional cross-talk between the host and the microbiota in the pathogenesis of a growing number of disorders, such as irritable bowel syndrome, inflammatory bowel disease, allergic diseases, and gastrointestinal cancer." In general, most scholars consider "that by 2 years of age, the microbiota resembles that of an adult, which is dominated in health and disease by species from only four phyla, *Firmicutes* (predominantly *Clostridia*; 50–70 % total bacterial numbers), *Bacteroidetes* (10–30 %), *Proteobacteria* (up to 10 %), and *Actinobacteria* (up to 5 %), with 90 % believed to be obligate anaerobes." For instance, in the work of Paliy et al. [96], "between 227 and 232 species were detected in fecal samples from children, whereas 191–208 species were found in adult stools" [96].

In conclusion, in a general state of health, the essential "gut microbiome acts as a highly efficient bioreactor, helping to extract energy and nutrients from the food we eat," but it also possibly has many "complex effects on human metabolism." As a consequence of these interactions, "changes in its composition have been linked" in addition to a number of diseases, also to "autoimmune disorders, and even diabetes and obesity" [91].

Chapter 9
The Global Microbial Environments

Although the word “environment” is of common usage in many contexts and discussions, there appears to be different manners of interpretation. Some use this word to signify the “biophysical environment” which corresponds to the “biotic and abiotic surrounding of an organism or population.” But this “can vary in scale from microscopic to global in extent” [97]. Therefore, the global environment can be viewed as the complete collection “of niches (or roles for different organisms)” and their “habitats (or different locations and environments for different organisms)” which are “necessary for the development” of many complex ecosystems on planet Earth [98]. From the point of view of microbial ecology of food pathogens, for instance, different types of environments are recognized “that for practical purposes can be divided into seven habitats types: water, food, soil, air, vectors, living reservoirs, and products of human activity” [99]. On the other hand, if we look at all the microbes and not pathogens alone that are associated with foods, “eight environmental sources of organisms to foods” are usually portrayed in order “to reflect their primary food-source environments”: “soil and water, plant and their products, food utensils, gastrointestinal tract, food handlers, animal feeds, animal hides, and air and dust” [100].

In all global environments, the smallest and most numerous organisms on Earth have been involved since the beginning of time, in all local and global processes. “The impact of microbes on climate,” for instance, is not new at all as it “goes back billions of years,” when the microbes “first evolved the genetic machinery for nitrogen fixation” and for “photosynthesis.” There is also a general agreement that “billions of years ago, changing microbial community composition resulted in the shift to an oxygenated atmosphere,” as “the organisms that had inhabited the Earth for at least a billion years were no longer able to survive on the Earth’s surface,” thereby “paving the way for the stupendous diversity of oxygen-breathing organisms that now inhabit our planet.” Microbes do not just live on most environments on Earth but they lead many processes and environmental changes. For instance, “until the development of industrial processes to fix nitrogen just a century ago, and with the

A. Zaccheo et al., *Food Hygiene and Applied Food Microbiology in an Anthropological Cross Cultural Perspective*, DOI 10.1007/978-3-319-44975-3_9

exception of a limited amount of atmospheric nitrogen that is fixed by lightning, all of life was dependent on microbes to convert nitrogen into usable form." Even to date, microbes are "critical players in the cycles of many elements." "Not only carbon, oxygen, and nitrogen, but also phosphorus, sulfur, iron, and many others" [53].

In regard to the current controversies surrounding climatic changes, microbes cannot by all means be disregarded. Because in the intricate microbial global food webs of this planet, "some of them are producing oxygen," others are using it, "some are taking carbon dioxide out of the air," others are expelling it, and the same goes for methane gas as well as other biogeochemical cycles. It can therefore be reasonably affirmed that many "major events of the distant past" seem to clearly "illustrate the need to incorporate microbial activities into existing climate models." Presently, "there is clear evidence that microbes can have an enormous impact on climate but their responses and impacts are currently unmeasurable." However, "we know that all of the microbial processes that affect climate do not necessarily balance each other out," and we are also cognizant of the fact "that microbial communities have a potential for rapid evolutionary change." This aspect "adds an additional layer of complexity to the task of predicting how community function will change as environmental conditions shift." "Today, changes due to human activity are causing similar large-scale global effects in as little as 100 years." Although compared to the natural fluxes of carbon dioxide through the biosphere, "the amount added by human consumption of fossil fuel every year might seem trivial. But over time, as the concentration of that gas has gradually risen, the physical consequences are now clearly measurable." What we know is that "in a changing environment, microbes and microbial communities can very rapidly respond and adapt." In turn "those adaptations have potential to feedback, either positively or negatively," and most importantly "there is not a second Earth that scientists can use for experimental purposes" [53].

Notwithstanding that a comprehensive discussion about microbial interactions in regard to climatic changes goes beyond the scope of this discussion and could almost inevitably lead to some strong debates and even polemics, there are however some clear indications of possible effects of current climate changes on the prevalence and incidence of some pathogens and parasites. For instance, "the unusually strong El Niño event of 1997–1998, followed by 2 years of strong La Niña conditions, the worst hurricane season on record," has coincided with "the reappearance of cholera in the Americas." Although climate is clearly "not the only factor" which can contribute to increased "risk of certain infectious diseases," because "demographic, behavioral, or socioeconomic factors may override any climatological relationship," it is also known that "in endemic regions such as Bangladesh, the incidence of cholera cases follows seasonal patterns." Furthermore, as cited by Rose et al., "Inter-annual variability associated with signals such as the El Niño-Southern Oscillation (ENSO) has been proposed to influence cholera outbreaks in Peru" by Colwell in 1996, and has also "been associated with dynamics of the disease in Bangladesh" by Pascual et al. in 2000 [101].

Additionally, extreme seasonal weather conditions have been shown to modify "the levels of anthropogenic pollution (including human viruses) in estuaries of south Florida," as it is generally recognized that "rainfall and runoff have been associated with individual outbreaks of waterborne disease," because "it is clear that pathogens of fecal origin can find their way into water." In this respect, a thorough "investigation in the USA" by Rose et al. [101] "has found that between 20 and 40 % of the surface water and ground water outbreaks from 1971 to 1994 were associated with extreme precipitation." Moreover, as suggested by LaBelle and Gerba in 1982, "while certain pathogenic or toxigenic microorganisms, including toxic phytoplankton and *Vibrio* spp., occur naturally in marine and estuarine waters," extreme seasonal weather conditions which are coupled with "anthropogenic contaminants including enteric bacteria, protozoa, and viruses may be introduced to coastal waters as sewage pollution." Whereas "despite the relatively unfavorable environment" for those pathogens, "these introduced organisms may survive for prolonged periods in the marine environment" and eventually make their way into our food supply [101].

Evidences to the importance of climate-sensitive diseases and parasites which are relevant to water and food safety seem to be rapidly mounting all over the world. Although the political debates surrounding the roles of humans in changes in weather patterns and climatic changes will most likely continue for decades to come, microbial organisms will not wait for our political and economic decisions and most certainly will continue to rapidly adapt and cease new opportunities for their survival and dissemination into available habitats. It should be also noted that the complexity of these situations will not only be caused by the direct effects of extreme weather on organisms, but will be compounded by the indirect effects on vectors and/or invasive species, which are also known to be very often associated with human mobility and transports of goods and merchandises as well [85, 101].

Chapter 10
The Local Food Environments

Humans for millennia, although most likely unaware of the existence, nature, origins, interactions, and diversity of microbial organisms, have been able to preserve many types of foods by applying such traditional "hurdles" to spoiling and pathogenic microbes as salting, drying, acidification, fermentation, osmotic pressure, oxygen exclusion, heating, chilling, natural preservatives, and more, either alone and recurrently in combinations among each other. History of human food preservation can therefore be regarded as the archetypical form of anthropic modification of countless "local microbial environments." Although "most of these methodologies were derived empirically" many years ago and most of them have been used traditionally for centuries, they are still very relevant today and constitute the wealth of "enormous diversity of traditional and artisanal methods for the preservation of foods" in the whole world. Customarily "the efficacy of the majority" of these methods in most perishable foods is not being used alone, but "depend on the use of multiple means for the inactivation or inhibition of contaminant microorganisms." "Since the 1980s," through the pioneering work of L. Leistner et al. at the German "Federal Centre for Meat Research (BAFF)," the science behind traditional foods has been not only elucidated but also refined by the understanding of *hurdle technology*, a term generally employed by scholars to describe the conscious and "intelligent use of multiple preservation procedures in combinations specifically relevant to particular types of foods." This integrated approach is designed for "the control of pathogenic, as well as food spoilage microorganisms, and to almost all food commodities and products" [102].

"Quality deterioration of foods is caused by a wide range of reactions": physical such as the movement of "moisture to or from the environment or between the components of a composite food"; chemical like in "rancidity caused by oxidation reactions"; enzymatic, like in "rancidity caused by lipolysis"; and microbiological which ranges from a simple limitation of the shelf life to "the presence or growth of infectious or toxicogenic microorganisms," which characterize many serious threats to the health of consumers. It is universally agreed that "the overriding priority" of

A. Zaccheo et al., *Food Hygiene and Applied Food Microbiology in an Anthropological Cross Cultural Perspective*, DOI 10.1007/978-3-319-44975-3_10

food-preservation technology “is always to minimize the potential for the occurrence and growth of food spoilage and food poisoning microorganisms,” by either delaying or preventing microbial growth and contaminations. All current and traditional techniques of food preservation are therefore designed to alter the microbial environments on food commodities and must operate through “factors” that are most effective in influencing “the survival and growth of microorganisms.” These factors were originally proposed by Mossel and Ingram in 1955, and later updated by Mossel in 1983, and comprise *intrinsic* and *extrinsic* parameters of all foods during storage and *implicit factors*, which are constituently “related to the nature of the microorganisms” in their interactions “with the environment with which they are in contact during growth,” as well as *processing factors*, which are actions specifically designed and “deliberately applied” to the food-microbial complexes with the goal of improving preservation. Lastly, there also *net effects*, which take into account that these “factors strongly influence the effects of each other,” so that the overall compounded or synergistic effect “may be usefully greater than the perceived effects of the single factors would lead one to expect” [102] (Table 10.1).

Table 10.1 Selected standard preservation methods in food-microbial environments

Processing factors	Selected examples
“Reduced temperature”	Chilling, cooling, freezing
“Increased temperature”	Thermization, pasteurization, sterilization[a]
“Reduced water activity”	Curing with salts, conserving with sugars, drying
“Decreased pH value”	Addition of acids, lactic acid, or acetic fermentations
“Decreased oxygen”	Vacuum and nitrogen packaging
“Increased CO_2 content”	Controlled or modified atmosphere packaging
“Addition of preservatives”	Inorganic, organic, biological compounds, some spices
“Decontamination”	Blanching, steaming, smoking, disinfecting (if allowed)
“Aseptic processing”	“Thermal processing and packaging” in closed systems
“Restricting of availability of nutrients” in emulsions	“Control of microstructure: compartmentalization of aqueous phases in water-in-oil emulsions” [102]

[a] Definitions of terms used by Leistner and Gould [102]:
“*Thermization*, to injure heat-sensitive vegetative microorganisms” [102]
“*Pasteurization*, to inactivate heat sensitive microorganisms” [102]
“*Sterilization*, to inactivate spore forms of microorganisms” [102]

Adapted from Leistner, L., Gould, G. W., ed. 2002. Hurdle technologies: combination treatments for food stability, safety and quality. Preface, 1st ed. New York: Springer Science + Business Media, LLC. p. 3

In regard to the table above, first it must be pointed out that the list is by all means not exhaustive because it excludes, for instance, many “new and emerging food-preservation technologies.” Second, most foods must undergo multiple preservation procedures during the standard preservation processing steps, as the concept of “hurdle technology” implies. However, the rationale behind repeating and adapting this commonly cited table by these distinguished authors is to demonstrate what the limits are surrounding some of the most generally axiomatic single methodologies, especially when they are confronted to the genetic diversity and to the environmental adaptability of microbes.

The importance of “low temperature” in food preservation cannot be underestimated, as it is always emphasized in all food safety classes. However, refrigeration reduces the “types of microorganisms that can grow” including many pathogens but not all. For instance, Graham et al. in 1997, reported that “Clostridium perfringens and the proteolytic types of Clostridium botulinum are unable to grow below 12 °C, whereas for nonproteolytic types of *C. botulinum* the lower limit is 3 °C.” Furthermore, some important “pathogens (e.g., *Listeria monocytogenes*, *Aeromonas hydrophila*, *Yersinia enterocolitica*) can multiply at temperatures below this, at near to 0 °C.” Although theoretically, a constant temperature “near to 0 °C” should provide “the possibility of highly effective and safe preservation”; the truth is that in “commercial practice, such precise control cannot be achieved for retail foods.” Also “below 0 °C, many nonsporing spoilage microorganisms are able to multiply slowly, probably down to about −7 °C, so that frozen foods may spoil slowly,” “even if thawing has not occurred.” Equally “below about −10 °C,” usually “no microbial growth will occur in frozen foods”; however, “occasional reports of slow low-temperature growth” have been observed for example by Collins and Buick, who reported in 1989 that the “growth of yeasts in frozen peas at −17 °C.” It is generally understood that “freezing reduces the water activity of foods,” and “since molds and yeasts tolerate lower water activities than bacteria,” “spoilage of frozen foods is more often caused by molds and yeasts than by bacteria.” It should also be noted that “chilling and freezing can be much better exploited in industrialized than in developing countries,” because in many “developing countries energy is expensive” and electricity is not always “continuously available,” as it is generally expected to be the norm in most industrialized countries. Furthermore, “climatic conditions,” in many developing countries such as prevalent high temperatures and humidity, “make the use of low temperatures in food preservation even more difficult” [102].

Heat treatments such as pasteurization and sterilization have been employed for decades in all “of the largest and most important food-processing industries.” “The heat resistance of the various types of microorganisms” is generally renowned to “cover an enormous range, depending on the particular species and sometimes on the strain, and whether the cells are in the vegetative or spore state.” Many vegetative cells such as the one of *Campylobacter* species are easily “eradicated from foods by the mildest of pasteurization regimes” in a “few seconds at 60 °C.” However, “some enterococci have heat resistances” “hundreds times greater” and can “sometimes survive and cause spoilage problems in pasteurized foods,” particularly if heated slowly, allowing time for the bacteria to rapidly adapt to heat, which can in turn increase their heat resistance from an estimated minimum threshold of “5 min at 60 °C up to as much 20 min at 60 °C.” Fungal spores and bacterial spores require generally much higher heat treatment than most vegetative cells. By comparison, spores of “*botulinum* type E” have a heat treatment range or “*D*-values of a few minutes at 80 °C,” while at the other end, “spores of *C. botulinum* type A have *D*-values as high as nearly 30 min at 100 °C” [102].

In most developing countries, heating process is the prevailing method “for eliminating undesirable microorganisms from foods.” However, “the heat treatment in these regions of the world is generally done at temperatures <100 °C since autoclaves” are not readily available. Fortunately in many of less developed countries, people are usually traditionally aware of the frequent "high load of microorganisms in the raw materials, caused by the climatic conditions and the lack of a cold chain." Therefore, the heat treatment to foods usually applied there "is often more severe than in industrialized countries," because tradition (and common sense) dictates that food should always be "thoroughly cooked." For example, street foods in developing countries like "many noodle shops" found traditionally on "street corners in Southeast Asia are, in spite of the hygienic conditions, microbiologically safe" so long as "the time between cooking and serving is very short." Likewise in China, during the traditional processing of dried meats, the process often includes a "heating step" so that there are only infrequent "food poisoning cases after consumption of such foods." In contrast, with dried raw meats such as the "African Biltong," which is usually "eaten in the raw state," some salmonellae problems can at times occur [102].

The processes of curing by adding salt, or conserving by adding sugar, whether they are used alone or in a combination all induce a gradual increase of the osmotic pressure with a consequent "reduction of water activity" in foods (a_w). Generally, at least some of the most common "spoilage bacteria, such as pseudomonads" are extremely intolerant to lowered water activity and cannot grow when the a_w-value is "reduced" around 0.97." By comparison, "*Clostridium* pathogens" are only inhibited at an a_w-value "below 0.94." While in contrast "most *Bacillus* species are inhibited at a value of 0.93," though some may also "multiply at a_w just below 0.90." "*Staphylococcus aureus* is generally known to be the most low a_w-tolerant" "food-poisoning bacteria," and it can multiply in conditions as low as "a_w 0.86 aerobically" and around "a_w 0.91 anaerobically." Furthermore, "many yeasts and molds" are also able to "grow at a_w levels below 0.86." Most "osmophilic yeasts and xerophilic molds" instead can grow "just above a_w 0.6." As a consequence of these survival ranges and water activities, most dried foods are usually formulated in such a manner to reach a target a_w-value approximately of 0.3, which has the additional advantage to "keep chemical and physical changes to a minimum." In many developing countries where food must be stored "without refrigeration", "most preserved foods are produced in the intermediate moisture range (a_w 0.90–0.60)" or "in the low-moisture rage ($a_w < 0.60$)." Local food manufacturers there, although they might be "generally not familiar with the concept of water activity" and/or lack the instruments to measure it regularly and precisely, very often do "preserve intermediate and low moisture foods effectively by following recipes which have been handed down for generations" [102].

pH reduction which corresponds to an increased acidity has been traditionally limiting the "growth of some important food spoilage and food poisoning microorganisms." Of particular importance is a pH value of 4.5, "below which *C. botulinum* is widely regarded as unable to grow." Generally, most if not all known "food-poisoning microorganisms are prevented from growing below pH 4.2." In contrast, Corlett and Brown in 1980, reported that many acid-tolerant food-spoiling microorganisms can grow at lower pH such as "lactic acid bacteria," and many "yeasts and molds" can grow "at pH below 3." In many developing countries around the world, "the pH is an important preservative hurdle in foods." In addition, probably because of some agroecological traits linked to conditions of high humidity and temperatures, some "prevalent foods there, especially fruits, have an inherent low pH." In many Western countries, some relatively acid meat products with a low pH (~5) are tolerated. However, in many Oriental countries such as China and Japan, "a sour taste of meats is associated with spoilage, whereas a sweet taste of meat products is appreciated." Therefore, especially in Oriental countries, there are only some "limited applications" where foods are also "preserved by high pH." As cited by Leistner and Gould (2002) in the review by Luo et al. in 1980, in China "the famous, traditionally preserved duck" and preserved "chicken eggs (*pi dan*)" sometimes called by Westerners "1000 years eggs," the alkaline preservation is achieved by treating these animal products with a "sodium hydroxide (NaOH) solution." In this regard, as explained by Meng et al. in the 1990s, the albumen treated with this strongly basic solution reaches a pH ~ 11 and the yolk around ~pH 9, which have the advantage to inactivate "high numbers of *Salmonella enteritidis* in and on the eggs" and also to coagulate the masses which "acquire no soapy taste, are ambient stable, and are eaten without heat treatment" [102].

In the last four decades mostly in industrialized countries, "there has been a substantial expansion in the use of vacuum and gas packaging." These technologies rely on removing oxygen for their effect. This consequently prevents "growth of strict aerobes" and also slows down "the growth of facultative anaerobes." As suggested by Molin in the year 2000, carbon dioxide (CO_2), "commonly incorporated into modified atmosphere packs," has been shown to exacerbate "additional specific antimicrobial effect that is particularly marked at low temperatures." The scopes of vacuum and gas packaging vary according to the characteristics of the foods. For example, in the case of "lipid-rich dried foods, such as fried potato crisps and similar snack products," although they are generally "microbiologically stable," they also draw organoleptic benefits "from oxygen-free packaging through the prevention of oxidative rancidity." Also during the "preservation of red meats," it is important to preserve the "bright red oxymyoglobin color," because it is usually associated with meat freshness, and also to suppress the growth of prevalent "Gram-negative bacteria during extended chill storage." Generally, a typical modified atmosphere mixture consists of "(70–80 % O_2:20–30 % CO_2)." However, in some other foods, such as salmon and carrots, studies have shown the "highlighted detrimental effects of high O_2 levels on product quality," through the detectable "early onset of oxidative rancidity." Furthermore, "vacuum packaging" is commonly and more generally used "for processed meats" such as *la chang*, which stands for the typical Chinese sausage in Mandarin, and *lap cheong* in Cantonese. In those instances vacuum packaging is generally considered "beneficial in several respects: rancidity and mold growth is delayed and flavor and color retention is fostered." On the contrary, in developing countries, "modified atmosphere-packaging is not yet widespread," mainly because of the "considerable investments and the regular gas supply needed" [102].

Practically in all of the industrialized countries, and more frequently in many developing countries, the common antioxidant ascorbic acid is becoming "readily available." Ascorbic acid addition in pure form or in natural extracts to many foods "further improves the color of the products and delays mold growth, because the redox potential is reduced." However, preservatives and sometimes acidifying agents and antioxidants, albeit some of these molecules, are either naturally extracted or identical to the ones commonly found in natural food ecosystems, and they are increasingly the subject of some controversies and sometimes even to polemical debates, more likely in some of the most developed countries of the world. It would not be possible to objectively discuss the pro and cons of many food additives in this context. However, some general principles can still be addressed. First, "the most widely used food preservatives" are mostly constituted by the "weak lipophilic organic acids (propionic, sorbic, benzoic)" and acidifying agents "such as acetic acid." These molecules are only effective if pH values are maintained low enough as to ensure that "substantial amounts of undissociated acids are present" in the substrate. Although there are several mechanisms involved, "a major effect of the undissociated acids is to act as "proton ionophores," which increase "the rate at which protons enter the cytoplasm." This mechanism gradually places the cell in a greater and greater demand to expel additional protons, so that the cell can maintain "homeostatically" a suitable high internal pH." Moreover, Bracey et al. have suggested as early as 1998, that "the degree of inhibition correlates well to the intracellular ADP/ATP ratio." As a result, as the microbial cells try to preserve a suitable pH, the "increased consumption rate of ATP" causes eventually the yield of proton expulsion to fall, so that when the "energy demands for proton extrusion" become excessive, the internal pH eventually collapses, and the microbial growth ceases [102].

"Sodium nitrate ($NaNO_3$) and sodium nitrite ($NaNO_2$)" are commonly used in curing meats, "because they stabilize red meat color, inhibit some spoilage and food poisoning organisms, and contribute to flavor development." NO_3 "has been shown to disappear on both heating and storage," "as many bacteria are capable of utilizing nitrate as an electron acceptor," thereby reducing nitrate to nitrite. "This ion is highly reactive and is capable of serving" both "as a reducing and an oxidizing agent." In acid environments, nitrite "ionizes to yield nitrous acid (3HONO), which further decomposes to yield nitric oxide (NO)." Nitric oxide in turn is the determinant factor for "color fixation in cured meats." Most importantly, nitrites are known to inhibit *Clostridium* pathogen "vegetative cells growth" and also prevent the "germination and the growth of spores that survive heat processing." Furthermore, in the case of cured air-dried products, nitrites are also known to act as excellent inhibitors of *Clostridium botulinum* and other relevant Clostridia. It generally appears that the principal antibacterial activity of nitrite seems to be caused by the "inhibition of nonheme, iron–sulfur enzymes," such as "ferredoxin." Therefore, this would in turn prevent the "synthesis of adenosine triphosphate (ATP) from pyruvate," thereby interfering with a key energy metabolic step in microbial cells. This preservative has been often implicated in the formation of highly toxic nitrosamines. Consequently, in the USA, for instance, in order to decrease "the potential hazard of *N*-nitrosamine formation in bacon," it was proposed to reduce dosage from ~100 to 120 ppm to a maximum of "40 ppm of nitrite" in a combination with "0.26 % potassium sorbate." This proposal was temporarily enacted for bacon in 1978, but was "rescinded a year later when taste panels" revealed other undesirable effects [103].

In regard to most preservatives, it should be pointed out that to date, with the notable exception of “parabens” or “*p*-hydroxybenzoic acid,” all preservatives depend on acidic environments, and there “are still no wide-spectrum antimicrobial preservatives that are effective at pH near to neutrality.” Presently, “in many developing countries” also, some “chemical preservatives” such as sorbates and nitrites are increasingly being used. In a rational cost to benefit analysis, this can generally be considered beneficial as long as the application’s dosage is correct. However, often this aspect especially in developing countries is “not sufficiently controlled,” principally by practitioners and by artisans, who might “overdose chemical preservatives,” either by lack of precise measuring and mixing instruments or by overzealousness. This practice should in any case be avoided for cautionary reasons. In some developing countries, e.g., in Africa, chemical preservatives, since they must be imported, are too expensive”, to be widely used. However, despite some apprehensions and disbeliefs by some, it has been demonstrated in several occasions that in “these conditions chemical preservative can be partially replaced by extracts of spices which are prevalent in these regions.” In fact, traditionally and over the centuries, an immense “treasure of knowledge of food-preservation methods” has been independently “accumulated in different regions of the world.” Surprisingly, some of “the processes employed for the preservation of foods in developing countries” are “largely based on the same principles as in industrialized countries.” However, the emphasis given to those principles seems to “differ considerably” with each region and culture. Usually for economic and practical reasons, “the variety of fermented foods” tends to be “larger in developing than in industrialized countries” [102]. For example, a recent “tentative risk profiling of selected traditional North African foods” was published which employed “as a template the “risk categorization model for food retail/food service establishments” developed by Health Canada.” This comprehensive review describes in detail many traditional North African foods, in regard to some of the most common combinations used there which include empirically “different natural hurdles to microbial growth” such as curing, salting, drying, fermentation, and antimicrobial spices [104].

The authors of this study also pointed out that “spices and herbs” added to traditional meat products in those areas such as “garlic, curcuma, cinnamon, cumin, ginger, cloves, paprika, and pepper (black, white, or red) coriander (leaves or grain)” can also “contribute to the improvement of food safety” and quality, “as many of them have been shown to possess potent antimicrobial activities.” Furthermore, “pre-treatments such as cooking or marinating into an acid, spicy preparation” not only improve the organoleptic quality of the finished product but the microbiological quality as well. Presently, as mentioned by Chaves-López et al. (2014) citing the patent request of Draganski A. in 2012, “local meat industries are trying to standardize” for instance the production of salty dry meat like *Gueddid* so its technology can become suitable for “an adequate transfer to industrial scale,” as it has been done in North America with jerky meat, in order to meet the “consumer demands and for export to other countries with high concentration of North African communities such as France, Italy, Spain, and Canada.” Also in better known products of Middle Eastern origin, which are produced in many countries of the world such as *pastirma* also known as *basterma* or *basturma* or more commonly in the West as *pastrami*, different "hurdles" for production have been successfully used for centuries. The term literally means applying "a strong pressing action," which is a necessary step during salting and curing, in which the meat is pressed to facilitate curing, expel the extra water, and facilitate uniform internal oxidations of the tissues. This step is also common to many cured air-dried products in the European traditions, such as *carne secca* and *Bündnerfleisch* and other similar products [104].

According to Leistner and Gould [102], this highly esteemed meat product, well known in both Moslem and Jewish traditions, is an intermediate moisture food product (IMF), usually "eaten in the raw state" and that can safely be stored for "several months at ambient temperatures even in warm and humid climates." Besides salting, drying, and pressing, "at least four additional hurdles are important for the stability" of this and other analogous products which "are nitrite, pH, competitive flora, and garlic." Traditionally in Turkey, the product is "preferably produced from September to November because flies are not prevalent during this season, the air temperature is not as high as in the summer, and the relative humidity is moderate due to scanty rainfall." Not surprisingly, this period of the year also coincides to the time of the year when most traditional European Mediterranean farmers would kill their pigs as well. In Italian, for instance, this process is called *la mazza* from the name of the sledgehammer, which was traditionally used to stun the pig in order to manufacture raw hams, salami, and many other renowned products. Logically just across the Mediterranean Sea, such cured meat products are generated by employing similar hurdles and also many common herbs and species, albeit in different dosages and proportions according to their specific recipes [102].

Mediterranean countries are also generally known for their olives and their olive oils. Long before vacuum machines were invented, in European, North African, and Middle Eastern countries and elsewhere around the world, oxygen exclusion in foods has been traditionally used for centuries, for instance, by means of covering vegetable products and other "foods with a layer of oil" or alternatively also with a layer of fat. Traditionally meat products such as dry hams are also produced through a number of hurdles, which include "nitrite, pH, competitive flora, and garlic." Furthermore, during pressing, seasoning, and maturing, different levels of oxygen exclusion also contribute to the typical color and the stability of these products. According to the availability of refrigeration facilities, foods are either prepared as "intermediate moisture foods" (IMF) with an a_w ranging from "0.90 to 0.60" or as "low-moisture foods (LMF) with $a_w < 0.60$." In many Asian, African, Latin American, and European countries, the general trend seems to be moving away from more traditional and stable methods of producing low-moisture foods because they are usually saltier or more sugary than intermediate moisture foods, which in turn it results on greater microbial risks [102].

Most traditional foods can be improved not only in hygienic and sanitary methods of production but also in respect to their hurdle technology before shifting as an example from low to intermediate or from intermediate to high moisture foods, in order to maintain stability and acquire more resistant to cross contaminations. For instance, the traditional Indian specialty "Tandoori Chicken," the "skinned chicken meat," can be processed first by being treated with a solution of "sodium chloride (3 %), sodium nitrite (0.012 %), glucono-delta-lactone (0.2 %), and starter cultures (*Lactobacillus plantarum*, 10^6 cf g^{-1})." Then "the meat is marinated for 24 h with spices and sour milk (8 %), and the pH reaches ~5.1." After, "the meat is fried to a core temperature of 82 °C, cooled, and mixed with a paste containing 5 % garlic and 0.1 % potassium sorbate." The technologically "modified tandoori chicken has a final a_w of ~0.86 and a shelf life of 7 days at 25 °C." This product when challenged through inoculation and survival studies by pathogens such as "*S. aureus, L. monocytogenes, Salmonella typhi*, and *E. coli*," as reported by Manish and Berwal in 1997, still "proved to be stable and safe" [102].

Garlic is an essential spice in many cultures from Mediterranean countries to China and in most countries of the world, where it has been used for millennia [18]. Besides being objectionable to taste and odor to some, it must be clearly pointed out that while on one hand garlic represents a traditional and a very important hurdle in the production of many cured meat products, known to inhibit "salmonellae inside the meat" and also inhibit several molds on the surface of many meat products, even if placed in conditions of "higher humidity and temperatures," caution should be taken because alone it is generally not sufficient to prevent spoilage organisms and pathogens. For its effect, garlic like most other spices in those perishable food systems must act synergistically during the fermentation for instance with salt and "lactic acid bacteria," as it implies in an hurdle concept. Generally when food is salted, lactic acid bacteria reaches very high numbers, and not only do they sharply "decrease the pH" around 5.5, but also "act as competitive microorganisms" against spoiling organisms and pathogens [102]. There are countless examples where a correct application in food microbiology "hurdle technology" has been successfully applied to improve and provide more stability and a greater margin of safety to many traditional foods, even if they have been produced for centuries. In most developed countries, for instance, both the relative prevalence and also the incidence of *Clostridium botulinum* in professional meat canning operations have been reduced drastically in the last decades. However, this pathogen continues to pose some important threats to the health of consumers in many "homemade preparations." The most important reason is that in modern operations where they apply correct hurdling methods, processing parameters are clearly identified and characterized so that "the germination of spores of proteolytic or group I strains of *Clostridium botulinum*" can be prevented by such cumulative conditions as "$pH < 4.6$; $a_w < 0.94$; NaCl of 10 % or more; $NaNO_2$ ca. 120 ppm; incubation temperature <10 °C; and a large aerobic bacterial biota" [105].

Seven years before discovering penicillin, "Alexander Fleming discovers that the enzyme lysozyme occurs in tears, saliva, and mucus" [24]. This antibacterial agent is also naturally contained in egg whites and milk and has been shown to be "quite effective against Gram + bacteria." Lysozyme is still commonly used in hurdle technology to control "late gas or "blown" defect "caused by clostridia" in many renown cheeses like Swiss, Edam, Grana Padano, and similar products around the world [106]. Yamasaki et al. have demonstrated in 1997, that lysozyme is effective in hurdle technology to prevent spoilage in acidic beverages by *Alicyclobacillus acidoterrestris*

[107]. In the case of concentrated milk and similar products like South American *dulce de leche*, these products are stabilized microbiologically by applying a combination of hurdles such as acidification, evaporation, heat treatment, and sugar [106]. Additionally in the case of the popular South American sweet *dulce de leche*, another hurdle is introduced in terms of "Maillard reactions," where the products of this non-enzymatic oxidation which are generated during the process of caramelization of sweet condensed milk" probably constitute an additional and "important hurdle" as well [102].

There are also great applications regarding hurdle technology in terms of the sanitation and stabilization of vegetable food products and drinks. For instance, Smid et al. have reported in 1996 that a compound extracted from cinnamon spice "cinnamaldehyde was found to be suitable for surface disinfection of tomatoes." Also Ejechi et al. demonstrated in 1988, that "the microbial stability of mango juice heated at 55 °C for 15 min" can be significantly "increased by supplementation with extracts of ginger and nutmeg" [107].

Chapter 11
Biocides and "Superbugs"

Before starting this discussion, it is useful to define some common terms used herein:

1. *Sanitizers*: products and "compounds that reduce the number of microbes without necessarily killing them or inhibiting their growth" [68].
2. *Disinfectants*: products "used for the destruction, inactivation, or removal of microorganisms" [68].
3. *Antibiotics*: "substances of microbial origins that in very small amounts have antimicrobial activity", which may include "synthetic and semisynthetic substances that are closely related to naturally occurring antibiotics and have antimicrobial activity" [68].
4. *Antibacterial agents*: products possessing either some *bactericidal activity* designed to "kill some bacteria" or alternatively with *bacteriostatic activity*, therefore, aimed at inhibiting "growth and reproduction of some types of bacteria" without necessarily killing them [68].

The term *biocide* combines the Greek root *bio* for life and "Latin" *cīda*, *cīdium*, and "derivatives of *caedere* to cut down" or the killing act (in compounds—cīdere) [108]. This seems to be the root of common words such as germicides, fungicides, insecticides, rodenticides, herbicides, and so on. Nevertheless, if we were to examine attentively and objectively the human food chains from a perspective "from farm to fork" [48], either in most animal or most plant production chains around the world, we will most certainly come across a staggering variety and different sorts of natural and synthetic "biocides." Biocides are generally molecules and organisms commonly used by humans purposely, in attempts to manage and control populations of different types of organisms [109].

Biocides can either be chemicals or biological, although some pest control methods also rely on physical treatments that inactivate or kill living organisms for instance with the treatment of seeds with heat. Heat is not generally referred to as a "biocide."

A. Zaccheo et al., *Food Hygiene and Applied Food Microbiology in an Anthropological Cross Cultural Perspective*, DOI 10.1007/978-3-319-44975-3_11

For the vast majority, biocides consist of single formulations or compositions of chemicals such as inorganic, organic, and biochemical molecules or biological toxins [110]. These chemical products can either be natural, semisynthetic, synthetic, and also recombinant. Some very successful biological biocides are also available such as bacteriophages, viruses, bacteria, fungi, insects, spider mites, and more. People often express some concern about persistent chemical biocide residues and their breakdown products in environment food and water and also on the detrimental effects of introduced and novel organisms. According to the principles of integrated pest management, most biocides if employed correctly, with regard to their environmental impact, toxic effects to humans and to other organisms, as well as residues in water and food, can be markedly reduced, often minimized, and if not eliminated. However, each situation that requires the “control” of living organisms with biocides should always be carefully appraised for costs and benefits and thoroughly evaluated for risks [111].

In all fairness, humans certainly did not invent the first biocides. At most, humans in the last few thousands of years, first by employing minerals and later by extractions from other living organisms, and more recently through chemical synthesis, have been trying to emulate other organisms in their abilities to synthesize various biocides. Many microorganisms have been producing secondary metabolites and a wide array of biocides for billions of years. Humans, in a few thousand years, have produced several thousand new biocides, some which have existed long before humans and others anthropogenic, albeit some of them are modified versions of exiting natural ones. Humans using biocides have a record of long and lasting successes, some minor and major failures, and also a few disasters [111]. Overall, the commonly used term “superbugs,” only a few decades ago, used to represent insects or spiders mites, which were becoming increasingly tolerant or resistant to human multiple applications of pesticides, creating a spiral behavior which entomology professor Robert van den Bosch described very eloquently as the “pesticide treadmill.” These days the term “superbugs” is generally used colloquially in reference to a different “treadmill” or “bug bomb”: the one of antibiotic-resistant bacteria [112].

Antibiotic resistance, however, certainly began long before Alexander Fleming discovered penicillin in 1929 from the mold *Penicillium notatum* [24]. “The beginning of the story” of β-lactam type of antibiotics like penicillin started a long time ago, when “the evolution of β-lactam production allowed organisms, including some bacteria, to destroy other bacteria,” ensuing “bacteria evolved β-lactamases to protect themselves,” and as a logical consequence, “additional β-lactams evolved,” and this long before humans acquired the “ability to use penicillins and cephalosporins as antibiotics.” In fact prior to Alexander Fleming, “bacteria developed countermeasures to many of these antibiotics, both before and after the pharmaceutical industry entered the picture.” The general evolution of antibiotics along with the consequent evolutionary steps of resistance very likely has “preceded the success of the pharmaceutical industry in β-lactam manufacture by a billion years.” It might sound disarming but we have to accept this simple fact: “the battle of bacteria versus the medical community is continuing” [113].

It is also important to recognize that antibiotic resistance "only holds clinical importance if it is related to the outcome of treatment associated with a pathogen." And especially in this context, of foremost importance is whether or not some "antibiotic resistance genes are transmissible" [114]. According to Arthur L. Koch, "when penicillin G was first used to treat infected people" by injection, "it was so valuable and amounts were so limited that it was recovered from the urine and reused." Then "penicillin V was developed; it was more acid resistant and could be taken orally." Therefore, as soon as the antibiotic molecule entered the contexts of the gastro-intestinal tract of the human microbiome, quickly "resistance became apparent." In fact, as early as 1940, Abramson and Chain, had already found that "some strains of Escherichia coli were already resistant" to penicillin [113]. In this context, it should be pointed out that clinical trials with penicillin did not start until 1940, when Howard Florey was able to use the penicillin "purified by Ernst Chain, assisted by Edward P. Abraham and Norman G. Heatley." In the meantime, "researchers at the US Department of Agriculture Northern Regional Research Laboratory" in an attempt to improve yields of penicillin "discovered that *Penicillium chrysogenum* is a better source of penicillin than the original strain" isolated by Fleming [24]. For these reasons, limited use of penicillin occurred until 1943, when "widespread population usage" began [115].

As soon as 1944, Kirby discovered "resistant strains of Staphylococcus aureus". although in the case of the first recorded *Escherichia coli* penicillin resistant, their "resistance mechanisms, in hindsight, were no doubt generated by the prior existence of β-lactamases present" in the environment. This is not the case after all of *Staphylococcus aureus*. Staphylococci, "originally only rarely had β-lactamases but coexisted in the same environment" with streptococci and other microbes and clearly "must have acquired the lactamases because of the lactam treatment." The consequence of human intervention with this antibiotic is that, rapidly, "staphylococci have acquired the *methicillin-resistant Staphylococcus aureus* gene" (MRSA), which is known to be "borne on plasmids," hence very mobile. Molecular genetic and phylogenetic evidences strongly suggest that "while the β-lactamases are all generally related, indicating that they all go back to the primordial penicillin-binding proteins" (PBP) from the preantibiotic era, some antibiotic-resistant genes such as the *mecA* gene[1] were "not maximally expressed because there was repressor control over its synthesis." This genetic regulatory mechanism implies that before human introduction of antibiotics, a "long evolutionary time was needed to create the gene and the controls for its action." Nonetheless, probably soon after that the antibiotics "methicillin and oxacillin" became "commercially produced and their medical use expanded," within this gene cluster the "regulatory genes were lost by inactivation or deletion," which had the advantage from a bacterial perspective to grant "resistance to stronger challenges to be mounted shortly after the beginning of the usage" of these antibiotics [113].

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[1] The *mecA* gene encodes for a penicillin-binding protein PBP 2′, which evolved from PBP in a distant past and is responsible for methicillin-resistant *Staphylococcus aureus* (MRSA) [113].

"In the late 1950s, several new β-lactams were discovered, such as cephalosporin C and 6-aminopenicillianic acid." These new compounds allowed "chemists to produce lactams that resisted some of the lactamases." Not surprising however "in hindsight, antibiotics including methicillin and oxacillin" were "associated with the appearance of extended-spectrum β-lactamases (ESBL) and cephalosporinases." Then again "in the middle of the 1960s, new plasmid-encoded β-lactamases appeared in Gram-negative organisms that had been subject to cephalosporins." "These enzymes have been designated SHV-1, TEM-1, and TEM-2. Of these, TEM-1 has caused particularly grave medical problems." These aspects stimulated in turn "the development of β-lactams containing clavulanic acid" and "penicillin-derived sulfones." This molecule "clavulanic acid irreversibly tied up the β-lactamase, thus protecting the β-lactam antibiotic, which was then able to inhibit" the penicillin-binding proteins "(PBPs) and block wall growth. In turn "new lactams" were developed "that were resistant to the β-lactamases like the carbapenems" and later "third-generation" cephalosporins" and many other compounds [113]. From the microbial side, "no radically new (de novo) resistance mechanisms had developed" in the last decades, but mainly preexistent genetic information were mobilized predominantly "by lateral gene transfers to pathogens from other organisms." One of the most important certain "effects of antibiotic use by humans was to lead to lateral transfer of resistance genes to pathogenic and agriculturally relevant microorganisms" [116].

Presently, the Centers for Disease Control and Prevention (CDC) report "Antibiotic Resistance Threats in the United States 2013" illustrates a "Timeline of Key Antibiotic Resistance Events" from 1940 to 2011. On the right side of this timeline, one finds the year and the name of the "antibiotic introduced" and, on the left side, the "antibiotic resistance identified" by the clinical literature. Just at first glance, one can immediately notice that in the examples presented, it took a few years or at best little more than a decade before some antibiotic-resistant bacteria to introduced antibiotics were reported in the specialized literature from their time of introduction [115] (Table 11.1).

Table 11.1 Year of introduction of selected antibiotics and first resistance reported

Active antibiotic compound	Year of introduction of antibiotic	Year of first resistance reported[a]	Difference in years
Penicillin	1943	1940	[b]
		“Penicillin-R *Staphylococcus*”	
Tetracycline	1950	1959	9
		“Tetracycline-R *Shigella*”	
Erythromycin	1953	1968	15
		“Erythromycin-R *Streptococcus*”	
Methicillin	1960	1962	2
		“Methicillin-R *Staphylococcus*”	
Gentamicin	1967	1979	12
		“Gentamicin-R *Enterococcus*”	
Vancomycin	1972	1988	16
		“Vancomycin-R *Enterococcus*”	
Ceftazidime	1985	1987	2
		“Ceftazidime-R Enterobacteriaceae”	
Imipenem	1985	1998	13
		“Imipenem-R Enterobacteriaceae”	
Levofloxacin	1996	1996	0
		“Levofloxacin-R pneumococcus”	
Linezolid	2000	2001	1
		“Linezolid-R *Staphylococcus*”	
Ceftaroline	2010	2011	1
		“Ceftaroline-R *Staphylococcus*”	

[a] “Pan drug-resistant (PDR) outbreaks (*Acinetobacter–Pseudomonas*)” are excluded
[b] “Penicillin was in limited use prior to widespread population usage in 1943”
Based and adapted from CDC, Center for Disease Control and Prevention, 2014. Antibiotic resistance threats in the United States, 2013 report [115]

It should clearly be stated, however, that in nature and especially when we take into account the staggering biodiversity and the numbers of bacteria, in addition to resistance genes, we also have to consider many intrinsic mechanisms, which results in differential susceptibility of microbes to antibiotics and other antimicrobial agents. In fact, from the very beginning of the “antibiotic era,” Alexander Fleming had immediately realized that penicillin was “most effective against Gram-positive bacteria and least effective against Gram-negative bacteria.” This condition is caused by intrinsic morphological differences in the bacterial structures of cell walls of these main groups of bacteria [24], because the Gram + cell wall is “composed of a relatively thick peptidoglycan layer and teichoic acid,” while Gram − cell wall is "composed of a thin peptidoglycan layer, lipoproteins, lipopolysaccharides, phospholipids, and proteins." This fundamental aspect, which can be ascribed to evolutionary mechanisms of survival, accounts for the differential results in Gram stain and retention or loss of "the primary stain when subject to treatment with a decolorizing agent" [68]. Interestingly, although this fundamental bacteriological method was introduced by Hans Christian Gram in 1884 and has been used since as "the most important method for separating bacteria into" these two groups, "the process by which Gram differentiation takes place" will later be explained by M. R. J. Salton in 1963 [24].

From an evolutionary perspective of earlier bacterial cells, "before transmissible plasmids and viruses arose," "resistance could only occur by mutation of the target genes of cells"; therefore, "transmission of a resistance gene from organism to organism was nearly or totally impossible." A number of such chromosomal resistance mutations are known today: resistance to rifampin by a change in the RNA polymerase; resistance to erythromycin, coumermycin, and streptomycin by modifying ribosomal proteins; and resistance to the inhibition of DNA gyrase by quinolones". However "in general this strategy is a poor alternative relative to lateral migration of a gene from cell to cell when both modes are possible." With streptomycin, for instance, which remains the first found aminoglycoside, "the first studied resistance," and also "the first demonstration of a genetic mutation in bacteria" results "from a very rare mutation that alters the protein S12 of the ribosome." However, according to Arthur L. Koch and Dykhusien (cited as personal conversation), "the S12 mutation makes protein synthesis considerably slower, but much less error prone." Therefore, in absence of streptomycin, the consequent fitness cost is that faster and less precise "wild type" will replace "the slower-growing mutant-resistant form." Unlike very mobile "plasmid-borne detoxification mechanisms," which instead move very rapidly "from unknown sites in the biosphere" and provide cosmopolitan forms of protection to pathogens "residing in an antibiotic-treated host," the "resistance mechanism by mutation of the S12 protein is of little clinical importance today" [116].

In the light of these observations, it can be naturally asserted that "human introduction of natural antibiotics," with the logical and consequent "selection for resistance mechanisms occurred in the target organisms" by few "broad categories" of mechanisms, namely:

1. "Reduced permeability"
2. "Inactivation of the antibiotic"
3. "Alteration of the target within the host"
4. "Development of a substitute (but resistant) biochemical pathway"
5. "Development of efflux pathways" [117]

In this respect, of particular interest in this context are also specific "efflux pathways of tetracycline," as well as the more "generic" efflux mechanisms "of a variety of quite different agents," which are important in "multidrug resistance" and in addition to the resistance of some disinfectants or antimicrobial agents [117]. Microbes have been very successful in responding and adapting to environmental pressures for billions of years, regardless of the origin of those stimuli. Among the many selective pressures over time, there are both natural and anthropic biocides, against which microbes have managed through "universal evolutionary responses" to behave "like moving targets" for at least one billion years and will certainly continue to do so in the future. Human antibiotics, however, are less than 100 years old. Nonetheless in such a short time, "medical researchers have discovered dozens of antibiotic compounds," which "harvested by scientists and dispensed by clinicians" who "did wondrous things for treating bacterial illnesses." For instance, "in the 1940s–1960s, infectious disease mortality dropped from close to 800 per 100,000 annually in 1900 to about 50 by 1960." Likewise, the global "life expectancy" increased by "56 % during the twentieth century" [118].

The reverse side of the medal is that the emergence and spread of antibiotic resistance is often inevitable. Evolutionarily is well known that "any force that reduces reproductive success in individuals from a population" as selective pressure will be faced against the biodiversity of the microbial world and their genetic diversity, which is characterized by large population sizes, rapid generation turnover, high mutation rates, and potentially frequent cosmopolitan lateral gene transfers. "Drug and pesticide treatments universally select for drug resistance," and "eradication is not always the best goal because pursuing that goal means maximizing selection pressures." Furthermore, eradication cannot be pursued if the pathogen has ecological reservoirs such as animals or insect carriers or multiple hosts, because the chain of transmission cannot be broken by isolation. In a dynamic and highly collaborative microbial world, "no class of antibiotics is immune to bacterial resistance." In general, science-based "management of a disease or infestation rather than eradication" tends to be "more effective at slowing the emergence and spread of resistance." Consequently, "treatment plans that incorporate evolutionary and ecological principles," aimed at containing microbes rather than a generalized "war on bugs," can also "deter or delay resistance," thereby "promoting patients survival and protecting economic investments" [118].

The generally proclaimed "concept of judicious or prudent use of antibiotics," however, does not apply as some suggest, only to "factory farms" and agriculture. But it applies to human medicine, veterinary sciences (e.g., pets), and phytopathology, and ultimately it is pertinent to every human being, especially where it is permitted to "self-medicate with over-the-counter antibiotics" or by other unsupervised means. Each of these activities clearly contributes as well "to the maintenance and/or amplification of resistance gene reservoirs." Moreover, since we can reasonably assume that most "transmission of antibiotic-resistant bacteria and genes" moves in every possible direction, "between animals and humans" and also between the environment and humans, antibiotics not only affect the pathogens but also commensal and beneficial organisms, which in turn can laterally transfer its resistance to pathogens. The frequently repeated recommendation that antibiotic use outside therapeutic purposes should be cut down can never be underestimated in its importance. However, whether or not "the restriction of the use of antibiotics in animals would lessen the burden of antibiotic resistance gene reservoirs” is still somewhat debatable for the meantime, since such hypothesis needs to be more systematically investigated in several different contexts [114].

On one side it is known that the “growth or survival of a resistant mutant in the absence biocide” has a fitness cost. However, in the absence of selective pressure from the given biocide, “the fitness cost can be reduced or eliminated by compensating mutations in the microbe.” This might not always be the case however, as suggested by Smith, et al. and Lipsitch, et al. in 2002. “Once the antibiotic resistance gene is initially selected (in animals), it can be maintained by transmission to bacteria found in humans with no further antibiotic use in that animal population.” In short, once the cat is out of the bag, or “once the cow is out of the barn,” closing the door or the bag “will not get them back.” It should be emphasized that these principles might also apply to some procedures of preharvest management as well, which in some instances should evaluate antibiotics usage to “lessen the potential for emergence of antibiotic resistance,” where this is necessary. Last but not least, in the case of water in particular, “the role of environmental contamination with antibiotics” and also with “other selective agents remains unknown and needs to be factored into any equation of selective influences for antibiotic resistance.” Notwithstanding that during the current debates on this subject there appears to be increasingly stronger claims that “the most certain means to avoid antibiotic resistance is to not use antibiotics,” this suggestion is probably neither realistic nor applicable in some contexts. For example, it is well known and accepted that “many plasmids and integrons encoding antibiotic resistance genes also encode resistance to heavy metals or quaternary ammonium compounds” which in turn could also become a selective factor even in the hypothetical absence of antibiotics applied by humans [114].

In conclusion we cite one of the many eloquent sentences by Arthur L. Koch: “the game will never be over in the chess match between bacteria, eukaryotic organisms, mankind, and medical scientists and engineers” [117]. Most scholars seem to agree that the current antibiotic resistance crisis requires some obligatory steps: “quantitative risk assessments,” “development of specific prudent use guidelines,” “surveillances,” and an “integrated management approach,” in all antibiotic applications. It seems also ethical and tenable to reiterate a reasonable and pressing recommendation made by many colleagues elsewhere to research institutions and politicians, which is to assign the research and development of new microbiome-based probiotics, vaccines, and to innovative integrated microbial management techniques, a higher level of priority in the near future [114].[2]

A further distinction must also be made here between antibiotics and other antibacterial agents. “An antibiotic must have the property that it acts against the microbe, and at least much less against the human or animal that is also simultaneously exposed to it.” While disinfectants, antiseptics, antimicrobial agents, and other destructive chemical compounds are designed to kill microorganisms in a

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[2] The current status as well as some risk assessments for selected antibiotic-resistant bacteria in different regions of the world were also examined in references: [119–142].

nonselective manner topically, antibiotics must act against specific microbes "and at least much less against the human or animal that is also simultaneously exposed to it". Their general usefulness depends therefore on having a wide spectrum of activity on target organisms on some specific substrates [116]. Contrary to some prominent claims, there are no "universal germicidal soaps" applicable contemporarily as detergents and biocides to all substrates and which have been shown to be effective on all microorganisms. However, since some antibiotic resistance genes are also known to encode for resistance to some germicides, the current antibiotic resistance controversies have prompted people and authorities "to take a closer look" at some antimicrobial and germicidal formulations as well [143]. Moreover, some attractive marketing allegations for some hand sanitizers and "germicidal soaps" are rightly under scrutiny at the moment, because they allegedly and ostensibly "carry unproven claims to prevent MRSA infections" or other ailments [144].

Although a thorough assessment on methods of cleaning, sanitation, and disinfection is beyond the scope of the present discussion, the foremost significance and importance to strictly adhere to specific national guidelines and recommendation for each product and the specific application cannot be overstated. In all fairness it must also be clearly told that all antimicrobial agents without exceptions have pros and cons, which may depend on their mode of action, the intended usages and application, or the generation of residuals and/or secondary compounds breakdown products, tolerance, or resistance. Besides, all of them have some limitations in their spectrum of activity against at least some microbes, which may originate from innate, acquired intrinsic, or extrinsic microbial characteristic. Similar to the situation with antibiotic resistance, however, the foremost concern should be whether or not these "sanitizing germicide resistance genes" (often linked to antibiotic-resistant genes) are also transmissible to other microbes through lateral genes transfer [114]. Lastly, we should not overlook the importance of biofilms in regard to food safety and spoilage, first, because biofilms are very hard to remove from most surfaces, and most importantly because it has long been recognized that biofilm accumulation on foods, food utensils, and surfaces increases the risks of "food safety and spoilage" [59].

It is rapidly becoming almost axiomatic that "when biofilms form on food processing surfaces, they exhibit resistance to common disinfectants." In fact, according to a search performed by the authors of a "comprehensive review on the paradox of mixed-species biofilms in the context of food safety" on "Pubmed" from 1978 to 2014, there have been approximately 23,300 articles with the keyword "biofilm" and roughly 3300 articles on "resistance to biofilms" published in the scientific literature during this time [145]. In this context, it should be noted that there is no single "magic silver bullet" against biofilms in all contexts and situations either. To date, chlorine is "widely used to sanitize drinking and swimming pool waters" in most countries as it has been successfully for decades [103]. In drinking water applications, chlorine provides rapid effects on most planktonic microbes and variable degrees of "residual activity" against most biofilms as well [32]. Chlorine-based compounds have been used for sanitizing many "food contact surfaces, work surfaces, and floor drains" and more. But there are limitations as well, especially that given by the enormous possible types of biofilms and microbial associations "outside the lab" which are available in nature and in agroecological complexes, the current complete arsenal of all antimicrobial agents seems at times rather impotent against some of the most tenacious microbial associations and communities and most single products [103].

In addition, in most known instances, microbial communities and foodborne pathogens develop biofilms directly on edible food surfaces. In these conditions some optimization of current techniques and an integration of classical and innovative or "alternative techniques" seem to be necessary, especially to decrease the food safety risks in ready-to-eat foods which by definition are consumed in their natural state without processing. Success has been achieved in some applications and conditions for instance by using a combination of known direct antimicrobial agents during washing of vegetables and meat substrates. However, such applications are not universally permitted, and, in addition, decontamination treatments such as washing and chemical disinfection may not reach those pathogens harbored and protected within the intrinsic impermeable structures of some substrates, like seed coats, for example. Furthermore, other considerations such as some possible adverse effect on food substrates must not be underestimated [103]. An alternative approach can be offered in some cases by indirect antimicrobials, which are commonly added to foods primarily for other effects than antimicrobial, and can operate as "multifunctional food additives." However, such as in the case of common "phenolic antioxidant" food additives, despite having received excellent reviews in the literature for specific applications, they are not universally effective, and they are also not allowed for some food applications by local regulations. Common additives, spices, oils, flavoring agents, natural organic acids, and other antimicrobial compounds have niche and potential applications as well, but cannot be universally used in many applications as well [103, 105].

We cannot disagree with many distinguished authors of mixed biofilm studies, suggesting that the research and development of innovative management techniques on biofilms such as the use of common "bacteriophages, anti-quorum sensing, bacteriocins, antibiofilms, essential oils, and surfactants," as well as the applications of biofilm "dispersal-promoting agents (DPAs)" and general enzymes such as "polysaccharide depolymerases, and esterases," and/or biofilm-degrading enzymes like "dispersin B," "proteases, nucleases, chitinase, and DNase," are all recognized to disrupt the exopolysaccharide matrix (EPS) of biofilms and can be reasonably account for some adequate level of "control mixed-species biofilms" as well. In a final analysis, these strategies seemingly appear to be more reasonable and "better alternatives to wage war against mixed-species biofilms" on food microbiomes in the long run, but economic considerations should also prompt us to find viable alternatives within and for traditional and developing countries as well [145].[3]

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[3] Antimicrobial activities and methods and current information regarding mixed biofilms in different regions of the world were also examined in references: [146–158].

Chapter 12
Food Safety Considerations About Selected Causative Agents

As human groups gradually progressed around 8000–10,000 years ago from “primitive” gathering and hunting societies to modern and “advanced” food producing societies, food supplies became more stably available, but concurrently some “problems of spoilage and food poisoning” also became manifest [16]. As human communities grew larger and domestication of animals became a more diffused practice, such proximities increased the probabilities of pathogens to be transmitted from humans to humans, as well as from animals to humans. Zoonoses, also known as zoonotic diseases, are the “inevitable consequence of human–animal relationships and interactions” [159]. Human, zoonotic, and environmental pathogens can be transmitted through various routes, but all causative agents of foodborne and waterborne diseases share the feature that they have to be ingested and transmitted exclusively or predominantly from either food or water consumptions [59]. Humans have developed many food preservation strategies over the centuries, most of which are still used today. However, as new preservation methods were discovered and refined, new and unexpected problems also arose. For instance, even if the production of “fermented sausages” had been used by ancient Babylonians and Chinese “as far back as 1500” BCE [16], Emperor Leo VI, the leader of the Byzantine Empire around 900 BCE, prohibited the “eating of blood sausage because of its association with a fatal food poisoning now presumed to be botulism” [24].

Although humans lacked any awareness of the existence of the microbial world, from the Paleolithic throughout most of the Neolithic, and until the germ theory of diseases gained general acceptance, surprisingly, humans somehow managed very successfully to modify microbial communities and interactions to their advantage. Objectively, the development of agricultural and modern societies could have never been possible without the help and the complicity of “humble microbes.” To date, many authors categorize microorganisms into “good bugs” and “bad bugs” [39]. But in reality, causative agents of foodborne diseases are not really “different from other life forms”, and they are neither good nor bad in our common and universal struggle for survival. From this perspective, familiar terms such as pathogen,

A. Zaccheo et al., *Food Hygiene and Applied Food Microbiology in an Anthropological Cross Cultural Perspective*, DOI 10.1007/978-3-319-44975-3_12

parasite, host, and prey need to be contextualized, because even if all living organisms exhibit marked differences in "size, complexity, and weaponry," ultimately, we all are "hosts and prey and parasites and predators" [160].

The first and most notable record of foodborne epidemics in Western civilization comes from the notes of the wars in ancient Greece by "Hippocrates and Galen" (ca. 460 BCE–200 AD) [24], where soldiers from the Greek campaigns were frequent "victims of dysentery." In fact, this disease has been known "to have influenced the outcome of major battles" of the past, through wars into the twenty-first century. The causative agents were identified "in the latter part of the nineteenth century" as bacteria of the "*Shigella* species." Currently, "the clinical disease entity of bacillary dysentery is used synonymously with shigellosis." This pathogen is typically transmitted in water and food via the fecal-oral route, but unlike many other food and waterborne pathogens, only "humans and higher primates" are natural hosts and reservoirs. The "genus *Shigella*" is divided into four species based on their serogroups: *S. dysenteriae* (serogroup A), *S. flexneri* (serogroup B), *S. boydii* (serogroup C), and *S. sonnei* (serogroup D). Recently, however, "studies on DNA relatedness" have shown that "*Shigella*" and *Escherichia coli* are so "closely related that they should be considered the same species" [161], and even if only a few strains among them produce enterotoxins, *S. dysenteriae* type 1 was also found to generate a Shiga-like toxin which is analogous to the toxins of *E. coli* O157:H7, which are known to cause very severe symptoms and consequences on susceptible hosts [66]. "*Shigella*" are considered among the principal contributors of diarrheal disease in most developing countries and globally even to date, especially with "young children," in "crowded conditions," where "inadequate water supplies and poor sanitation" subsist, such as in the cases of the urban poor, migrant workers, natives, and inside inadequate or cramped institutions [161, 162].

Long after ancient Greece and the Roman empires were relegated to history, Pope Urban II declared the First Crusade in 1095, causing thousands of people to move across the Mediterranean regions. But as crusaders returned from the Middle East, they also brought back an emerging infectious pathogen unknown in Europe at the time: "typhoid fever." The germ rapidly spread throughout Europe and became "epidemic in 1480 and 1481 in Germany and France and in 1489 in Spain." After the rediscovery of the "New World" in 1492 by Europeans, the typhoid bacterium was carried by travelers to the new continent. The Jamestown colony was "founded in Virginia by English explorers" in 1607. But suddenly in the community, "more than one-half of the 105 persons who landed there" died "during the summer of the same year," and in the following years until 1624, "6454 out of 7549 colonists" also perished. The reasons for this hecatomb with a shocking 85 % death rate are controversial, possibly because "the period of 1609–1610" is commonly "referred to as "the colonist starving time," leading some writers to believe that beriberi (vitamin B1 deficiency)" was the cause of those deaths. Other authors have disputed, however, that the "symptoms described are more likely those of typhoid fever, possibly brought by a passenger on a ship from England" [24]. In any event, the pathogen would have been most detrimental on those colons already weakened by scarce nutrition and who lacked any adequate care at the time [66].

Typhoid fever (and other infectious diseases) ravaged many populations around the globe for centuries, but few clues and ideas regarding their spread were applied for a long time. Notably, however, William Budd in 1856 intuitively first suggested "that the infectious agent of typhoid fever passed from infected person in feces and is contracted by other individuals through contaminated drinking water." The author without experimental data to support his hypothesis proposed to disinfect water with disinfectants such as "chlorine water, chloride of lime, and carbolic acid to control infection." He published in 1873, *Typhoid Fever: Its Nature, Mode of Spreading, and Prevention*, which gradually grew a wider acceptance in spite of the resistance of many physicians and hygienists at the time. The "typhoid bacillus" was first observed (but not cultivated) by Karl Joseph Eberth in 1880 and was originally named *Eberthella typhi*, until its current classification in the genus *Salmonella* was applied [24]. The *Salmonella* genus "was coined in 1900 by Lignières," in recognition of Daniel Elmer Salmon and his assistant Theobald Smith, who pioneered microbial and immunology of *the hog cholera bacillus*[1] [163]. Typhoid fever is linked to two serotypes: "*Salmonella* Typhi and *Salmonella* Paratyphi A," both of which, unlike other known *Salmonella* serotypes, are restricted to the human hosts [66].

Vaccination against smallpox viruses had been used in China since ancient times. In 1776 the "commander in chief of the rebellious American Continental Army against the British" George Washington (who starting from 1789 also served as the first president of the USA) rightfully required "his entire army to be inoculated against smallpox" with pussy material, sparing his personnel from this debilitating disease. In contrast to the small pox viruses which generate pussy material on the surface of the skin that can be used as an inoculum, a vaccine against typhoid fever required the isolation of a pure bacterial culture, and this was not feasible until the development of aseptic methods and plating techniques. In 1884 one of Robert Koch's assistant Georg Gaffky isolated for the first time the typhoid germ. Shortly after, in 1898, Almroth Wright began his field trials with a vaccine on "British troops in India" and in South Africa "during the Boer War." In 1901 "after successfully testing a killed vaccine for typhoid fever, the U.S. Army makes typhoid vaccination compulsory" for all military personnel. And, from 1904 to 1909, William Boog Leishman's improved method of preparation of the vaccine, following his "successful tests in India that lead to broad use of the vaccine in World War I" [24].

Typhoid fever gradually became a lesser threat in most developed nations around the turn of the twentieth century, before the beginning of the "antibiotic era." Not only because of the vaccines, but mainly because of the implementation of general hygiene principles, which lead to a sharp reduction of fecal contaminations of water and food supplies, through the "disinfection of drinking water, sewage treatment, milk sanitation and pasteurization, and shellfish bed sanitation" [164]. Interestingly, however, scientists at the time were not yet aware that enteric pathogens can be

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[1] Interestingly, *the hog cholera bacillus* was later found to be of "viral etiology" instead, because bacterial cells were killed but not removed from the inoculum [24].

carried in a covert infection state, without developing any manifest symptoms on seemingly healthy carriers [163]. Strikingly in 1903, Mary Mallon, the first known "asymptomatic carrier of typhoid fever," was "implicated as the source of a series of typhoid outbreaks in New York City, in establishments where she was working as a food handler. The woman, aka "Typhoid Mary," was "placed under detention in 1915 until her death in 1928," at the age of 69, for having failed "to respond to repeated requests to cease working in kitchens" [24, 165]. But asymptomatic chronic typhoid carriers, however, are not uncommon and comprise approximately "2–4 % of patients infected with *S. typhi*." In those particular instances, "the pathogen remains deeply seated in the gallbladder, liver, or biliary tree," and it is "excreted periodically," particularly if the carrying host is subjected to stress. It is not completely clear, however, why asymptomatic carriage seems to be more frequent with "females and elderly people, with a median age of 60 years" [163].

Typhoid pathogens are still a significant source of morbidity and mortality in many parts of the world to date, particularly "in poorer nations," and most significantly with young children [162, 166]. Interestingly, as typhoid fever gradually became less prevalent in many parts of the world because of improved sanitation; non-typhoidal salmonellosis has steadily and continuously progressed all over the world "decade by decade since World War II" [164]. Notably also, up until a decade ago, it was believed that *S. typhi* was more common and widespread than *S. paratyphi*, but this assumption "may no longer be true, especially in southeast China," where *S. paratyphi A* is presently being more frequently isolated than *S. typhi* [166]. This constitutes a serious problem, because recommended vaccines against *S. typhi* already do not exempt vaccinated people from following good hygiene practices. But most importantly, these vaccines cannot provide useful protection against *S. paratyphi A* and might therefore gradually become less useful in Asia until a bivalent vaccine is developed, licensed, and distributed [166, 167].

In 1768, an environmental bacterium of "brackish water, estuaries, and salt marshes" "present in the subcontinent of India since ancient times" [168] suddenly became epidemic and in one single year "kills as many as 60,000 people" in India [24]. A few decades later, as trading and commerce between India and surrounding areas started to expand, the pathogen rapidly propagated to nearby geographical areas and quickly earned global recognition in the first cholera pandemic of 1817 [168]. Starting from India, the contagion propagated to "East Africa, Asia, Japan, the Philippines" [24]. The pathogen spread to China (1820), Arabia (1821), Japan (1822), and to the European territories (1822–1823). The first wave progressed a few years, but after a brief respite [168], a new epidemic began from Southeast Asia in 1826 [24], leading to the second cholera pandemic of 1829, when the germ spread even farther [168], reaching Poland, Germany, and Britain in 1831; Paris in 1832; and Canada, carried by Irish immigrants. From Canada, the pathogen spread to New York City, hence, rapidly expanded both west and south of the USA [24]. Typical control measures consisted mainly on "quarantine of infected individuals, maintenance of good personal hygiene, and adherence to methods of general cleanliness of water supplies, streets and environs" [168].

After its arrival in 1831 in Britain, cholera caused several sporadic outbreaks. During one of such outbreaks in London, John Snow in 1849 presented “his first observations on the transmission of the disease cholera through drinking water,” where he hypothesizes a connection between the disease and the contamination of drinking water by sewage. For his efforts he “is awarded a prize of 30,000 francs by the Institute of France” [24]. In his essay he first suggested “that cholera was caused by the ingestion of living organisms that could not be observed with microscopes available at the time.” And as the year of 1852 marked the start of another and third cholera pandemic from India [168], the germ rapidly swept across the whole European continent without restraints. In 1854, Filippo Pacini first described the cholera bacillus and named it *Vibrio cholerae*. And the following year, John Snow published his research *On the Mode of Communication of Cholera*, where he provided quantitative data on raw sewage contamination of “the Broad Street water pump (now Broadwick Street in Soho),” determining it to be the main source of the outbreak, in what is now considered to be “one of the earliest thorough epidemiological studies” on the spread of enteric diseases [24]. In his essay, John Snow correctly concluded from his data that “cholera was communicable from person to person and that it was transmitted by the ingestion of tiny amounts of infectious fecal material that contaminate foodstuff or water” [168]. In 1858 Florence Nightingale published her notes on the strict hygiene principles she had applied at her hospital during the Crimean War, which markedly reduced the frequency of “deaths from cholera, dysentery, and typhoid” of British soldiers under her care [24].

But in 1863 cholera spreads from the Ganges river in India once more and expands “to ports throughout the world,” reaching “the west coast of South America” for the first time. By 1873, however, the “US public health officials” had recognized the fact “that cholera is spread through fecal matter in water.” And as a result of the measures of improved sanitation which were taken, “the disease’s appearance in 1873 in New York, New Orleans, and other port cities” remains the “last outbreak of epidemic cholera in the USA.” Meanwhile in Europe, Robert Koch after isolating pure cultures of *Vibrio cholerae* during his expedition in India (1884) [24] demonstrates beyond any reasonable doubt that the pathogen can induce cholera, by inoculating it in the duodenum and in the gastric system of guinea pigs[2] [168]. In 1892 Robert Koch, upon the request by the local authorities during a “severe outbreak of cholera” in Hamburg, in spite of the very strong objections from eminent hygienist Max von Pettenkofer, still “insists on quarantine and isolation to help control the spread of the disease.” Ironically, however, even as Koch’s methods are proven to be successful in curving the epidemic, stubbornly Max von Pettenkofer just to prove his conjecture that the germ in groundwater must first undergo “changes leading to miasmas that cause the disease” deliberately drinks a pure “culture of the cholera bacillus,” obtained from Koch’s colleague Georg Gaffky, whereby he suffers from vigorous “diarrhea over the next several days but recovers without further incident” [24].

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[2] The guinea pigs were pretreated with sodium bicarbonate and injected in the peritoneum with a tincture of opium to prevent reverse peristalsis [168].

The path to a cholera vaccine starts in 1880 with Louis Pasteur and assistant Émile Roux, reporting that attenuated (less virulent) chicken cholera cultures (*Pasteurella multocida*), obtained "by holding them for several weeks at room temperature in the laboratory," offered protection against infection to the inoculation of the chicken with virulent strains. In 1885 Jaime Ferran y Clua administered a vaccine with a room temperature attenuated living bacteria to "approximately 30,000 persons," causing "severe adverse reactions in many of his subjects." Immunization attempts in India in 1892 with heat attenuated germs by Waldemar Haffkine were found more effective, but since "too many side reactions" still occurred, the vaccination trials were suspended. In 1896, Wilhelm Kolle instead prepared and tested an effective "heat-killed cholera vaccine using cells grown on agar re-suspended in saline," which was successfully used for many years [24]. Notably, however, to date, all of the licensed cholera vaccine cannot offer complete protection, and "vaccination should not replace standard prevention and control measures" [169].

We are presently in the seventh cholera pandemic, which began in Indonesia in 1961 and has lasted so far longer than all of the previous cholera pandemics: that is, more than 50 consecutive years in many parts of the world [170]. Cholera is endemic in more than 50 countries worldwide [171],[3] and it is ranked 7th among the 17 most important pathogens worldwide, in terms of "disability-adjusted life years for each pathogen acquired from contaminated food" [162]. Under the current taxonomy, the genus *Vibrio* comprises 28 species, "10 of which may cause illness in humans" [168]. Historically "serogroup O1" was responsible alone for "epidemics and pandemic cholera outbreaks." But all of the sudden in 1971, the pathogen propagated in a new epidemic wave "to Asia, the Middle East, Africa, and parts of both eastern and western Europe" [24]. Since then, evidences have been mounting that the El Tor biotype has "replaced the *V. cholerae* classical biotype worldwide." This biotype is often "associated with more prolonged clinical outbreaks," characterized by different and multiple waves of infections, and "mild disease or short-term asymptomatic passage," which are difficult to detect and facilitates its "silent introduction" in new areas. Besides, in the early 1990s, this biotype has "mutated to a new serogroup O139," which has rapidly spread to different countries in Asia "joining O1 as a cause of epidemic cholera" [170].

In 1990, El Tor biotype that began a new epidemic in Peru, probably starting from the harbor of Lima, believed to have originated from the discharge of some "contaminated bilge water from a ship from eastern Asia," which in turn contaminated fish and shellfish there, causing 366,000 cases of cholera, killing nearly 4000 people in one single year. By this time, however, the spread of the pathogen to other countries was limited to air travel of infected individuals and "fruits and vegetables exported from South America" [24, 172]. Most dramatically in 2010, in Haiti following a "catastrophic earthquake," an outbreak of cholera was swiftly observed, where "for the first time in more than a century," 665,000 people were infected and

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[3] WHO, World Health Organization, 2014. *Cholera, areas reporting outbreaks, 2010–2013.*

[Website] Available from: http://gamapserver.who.int/mapLibrary/Files/Maps/Global_Cholera_outbreaks.png [05.07.2015] Reference [171].

8183 people died. This dramatic toll in human lives, however, could have been much higher if prompt international intervention, aids, and surveillance had not been put in place rapidly after the emergency occurred [173–175].

The epidemiology of foodborne diseases is rapidly and continuously changing at a much faster rate than science and technology. And even when a pathogen may appear to have been “almost conquered” and “almost forgotten,” new foodborne illnesses are identified either as “newly recognized pathogens emerge” or as some “well-recognized pathogens increase in prevalence or become associated with new food vehicles” [176]. For instance, around World War I as a result of improved sanitation, typhoid fever sharply decreased in importance in many parts of the world. But in contrast, the global impacts of non-typhoid salmonellosis strains “have increased decade by decade since World War II” [164]. One of the main reasons for such a shift is the remarkable biodiversity of *Salmonella*, whereas from the original 44 known serotypes classified in 1934, it has climbed to over 2500 of them to date. Nevertheless, for reasons which are yet to be clarified, only a minority of them, around 20–30 serotypes, are involved in the vast majority of human infections [66, 163, 177–179]. Over time, variations in the frequency of specific serotypes occur, as some serotypes may increase in their frequency while others may decrease, as shown by a detailed laboratory-based enteric disease surveillance data in the USA, from 1968 to 2011[4] [178]. In the European Union for comparison, among all of the known reported human *Salmonella* cases in 2012, the most frequent serotypes were *S. enteritidis* (41.3 %), *S. typhimurium* (22.1 %), monophasic *S. typhimurium* (7.2 %), and *S. infantis* (2.5 %) [179].

Non-typhoid *Salmonellae* survives on many habitats and specific animal reservoirs [163]. Foodborne outbreaks caused by *S. enteritidis* are “most commonly associated with the consumption of contaminated eggs and poultry meat,” where it “may be transmitted both horizontally and vertically” in breeding flocks, with no apparent sign of infection [179]. *S. typhimurium* in Europe is “mostly associated with the consumption of contaminated pig meat or bovine meat” [179], but it is also found on vegetables and a “wide variety of food commodities” worldwide [177]. Globally, non-typhoid *S. enterica* was ranked in 2010 as the first and most important foodborne hazard based on “disability-adjusted life years at the global level,” followed by typhoid pathogen *S. typhi* [162]. Many countries have implemented different non-typhoid *Salmonellae* reduction programs, which have reduced to some extent the rates of salmonellosis in humans [179, 180]. However, since these pathogens can survive in ponds and sediments, in frozen and dry substrates, and develop themselves on different animal reservoirs, ranging from domestic to wild, from warm to cold blooded animals, as well as protozoan cells, their eradication from all food chains is unrealistic [163].

Currently known enteric bacterial pathogens like non-typhoid *Salmonella* spp., typhoid *Salmonella* spp., *Campylobacter* spp., Shiga-toxin producing bacteria, entero-

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[4] Atlas of *Salmonella* in the United States, 1968–2011: Laboratory-based Enteric Disease Surveillance. [Website] Available from: http://www.cdc.gov/salmonella/pdf/salmonella-atlas-508c.pdf [14.06.2014] [178].

pathogenic and enterotoxigenic *E. coli*, *Shigella* spp., *Vibrio cholerae*, and *Brucella* spp. [162] are highly adapted to survive in the gastrointestinal tract of humans and animals. However, unlike all recognized foodborne enteric viruses and most foodborne parasites, which are linked directly to "human and animal fecal matter," most enteric bacterial pathogens can also replicate in the environment and thrive on aquatic and farm environments. Hence, "once introduced to a food production environment," they may also "proliferate, making them difficult to control" [181]. Furthermore, a common term such as *infection* takes profoundly different connotations when it comes to enteric bacterial pathogens, because in enteric bacteriology, *infection* only specifies the "colonization of the intestinal tract," regardless of whether the hosts might be "symptomatic patients" or rather "asymptomatic carriers." Conversely within such a complex system involving host-pathogen interactions and food matrixes [182], there are conditions in which "almost any bacterial species is capable of producing intestinal symptoms if swallowed in sufficient numbers" [183].

Foodborne outbreaks "occur whenever pathogenic agents in sufficient number or quantity encounter a susceptible population without effective interceptive measures," and if we did not expect it to happen, we conclude that it "emerged" [160]. For instance, *Listeria monocytogenes* was known by the medical community as one of the causative agents "of meningitis and other invasive infections in immunocompromised hosts" for almost a century. But "how these hosts became infected remained unknown until a series of investigations identified food as the most common source" and finally acquired the full status of a foodborne disease [164, 184], in 1981, in an outbreak in Halifax, Nova Scotia, involving "pregnant women and neonates" and "linked to the consumption of coleslaw" [185]. *Campylobacter jejuni* was also known as a "rare and opportunistic bloodstream infection, until a veterinary diagnostic method used on specimens from humans showed it was a common cause of diarrheal illness" [164]. But it took decades before it acquired the full status of a major food pathogen, and it is now linked to many foodborne outbreaks in different food commodities all over the world [186]. Based on "disability adjusted life years for each pathogen acquired from contaminated food," *Campylobacter* is currently ranked as the sixth most important pathogen worldwide [162].

Interestingly "many of the pathogens of greatest concern today" were not "recognized as causes of foodborne illness" until just a few decades ago. And while technological advances have almost eliminated some diseases, even in those countries which generally enjoy the benefits of improved sanitation, "new causes of foodborne illness have been identified" [187]. As a result, many more additional commodities which were previously thought to be "safe" are now considered to be "hazardous." By extrapolation, "at the rate at which new pathogens have been identified, suggests that many more remain to be discovered" [164]. And even if our knowledge progresses fast, microbe's minutes are "comparable to human years." In ultimate analysis, we simply have to face the fact that microbes are "opponents with whom we cannot race—on their terms" [159].

Part III

The Elements Interacting with Each Other: Conclusions and Recommendations

Chapter 13
Conclusions and Recommendations for Those Outside or Inside the "Global Village"

Humans have traveled and traded goods over great distances since ancient times, and at what point trading began to assume a global connotation is disputable. Some scholars feel that trading could have already been considered "global" 2000 years ago, during the Silk Road. Others contend that the beginning of a global trade was marked by two main events of the fifteenth century: when Cristopher Columbus stumbled "on the Americas in search of spices" in 1492 and when Vasco da Gama successfully made "an end run around Africa" to circumvent the Arab and the Venetian monopoly on the spice trade in 1498. In contrast, according to O'Rourke and G. Williamson, the economy in the fifteenth century was still highly fragmented, and trading became globally integrated in the nineteenth century [188]. This epoch coincides with the first cholera pandemics as well, but in reality the human-enhanced microbial globalization had begun much earlier, because humans have been inadvertently transporting plant, animal, and human pathogens to and from distant places since ancient times [24, 161, 165, 168, 189]. Historically, microbes used to travel around the world faster than the information about them. But in the last century, an increasing proportion of people have interconnected, first through the radio and the television and more recently through the World Wide Web, which have virtually eliminated the effects of space and time in information, as if humans had contracted into a metaphorical "global village"[1] [190, 191].

But this metaphorical "global village" is not as harmonious and peaceful as some of the "popular use of the term would suggest" or as Marshall McLuhan had originally wished, because as he pointed out, life in the "global village" has its shadow sides as well [191]. For example, among an estimated human population of 7.3 billion people [83], 1.2 billion people live with no electricity and are denied access to the "global village." 2.7 billion people do not have "clean cooking facilities" to prepare

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[1] The term "global village" was coined by Marshall McLuhan in the 1960s, "as a way to describe the effect of radio in the 1920s in bringing us in faster and more intimate contact with each other that ever before in human experience" [190, 191].

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their foods [192]. 2.5 billion people have no access to "improved sanitation facilities" to fill their basic physiological needs. "Seven hundred and forty eight million people lack access to improved drinking-water," and one billion people, "nine out of ten in rural areas," practice open defecation. An estimated 1.8 billion people use sources of drinking water contaminated by feces, and "hundreds of millions of people have no access to soap and water to wash their hands" [193]. So, realistically, food safety must be regarded as "a privilege of the wealthy," because in many parts of the world "where 1.25 billion people live on 1–2 dollars per day, achieving an adequate food supply takes precedence over food safety" [189].

And even if some situations for the less privileged have slightly improved in the last two decades, since 2.3 billion more people have gained access to "improved drinking water" from 1990 to 2012, the global number of children dying from diarrheal diseases, which are "strongly associated to poor water, and inadequate sanitation and hygiene," has declined from 1.5 million deaths per year, to 600,000 deaths per year in the same period [193]. These success stories only partially alleviate existing profound inequalities. The estimates by the Foodborne Disease Burden Epidemiology Reference Group (FERG) regarding the 31 main global known pathogens show that "the burden of foodborne diseases is borne by individuals of all ages, but particularly by children under 5 years of age, and by persons living in low-income sub-regions of the world." Overall, one third of the deaths caused by foodborne diseases are children, and one third of the global toll for food foodborne diseases are in the African region. More than half of the morbidity and mortality cases caused by typhoid fever and hepatitis A virus occur in the Southeast Asia region. And 70 % of the diseases and deaths are caused by aflatoxins, and the highest global death rates caused by foodborne parasites occur in the Western Pacific region [162].

In lower-income subregions, an interrelated and mutually reinforcing set of problems, caused by poverty and by malnutrition, contaminated food and water, parasites, and diseases, perpetuates conditions, which interact synergistically to maintain "foodborne diseases at a high level" and enhance "the selection of new and more dangerous pathogens" [160]. Furthermore, hot and humid climates, associated with improper handling and storage of foods, combined with the current changes in precipitation patterns, extreme seasons, and natural disasters, all contribute in providing conditions which are conducive to abundant growth of toxicogenic fungi on crops and their consequent impact on human health [70, 160]. Humans are currently exerting a pressure on the biosphere to an unprecedented level, and since "almost every ecosystem on the planet bears the imprint of human influence," "these impacts extend to the microbial members of those ecosystems." But "in the face of profound and ongoing changes to the planet, we are still equipped with distressingly limited abilities to predict the response of microorganisms even in the most common-place, and seemingly simple, systems" [50].

Anthropic destruction of wetlands reduces the capacity of ecosystems to absorb nutrients, and deforestation decreases the infiltration of water into soils, which negatively impacts the replenishment of ground waters, reduces fresh water resources, and increases flooding. And all these activities, associated with intensive animal and plant productions, increase the nutrient content of water ecosystems [32]. Excessive nutrient content, in turn, destabilizes microbial communities and promotes the growth of toxic cyanobacteria, which can poison drinking water supplies and adversely affect terrestrial and water organisms as well [194–196]. Excessive nutrients in coastal sea waters and oceans, in turn, upset balances that "keep harmful algal blooms in check," and toxic algae poison humans and wildlife that consume shellfish or fish tainted by these organisms [56]. Moreover, eutrophication of the water ecosystems also harbors, protects, and favors the reproduction and the transmission of bacterial pathogens, such as algal blooms preceding cholera epidemics in Bangladesh, for instance [32].

Climate is not the only factor in the spread of toxins and infectious diseases, because interacting elements of "demographic, behavioral, or socioeconomic factors may override any climatological relationship." The inter-annual variability in climate which is "associated with signals such as the El Niño-Southern Oscillation (ENSO) has been proposed to influence cholera outbreaks in Peru," "has been associated with dynamics of the disease in Bangladesh," and is correlated to "the levels of anthropogenic pollution (including human viruses) in estuaries of south Florida" [101]. Interestingly, microbes seem to remind us that the planet is one and highly interconnected, because, even if poor water quality is typically associated to "developing countries," these regional distinctions in water safety can be arbitrary, since "intra-urban health differentials can be at least as extreme as those between countries." And current water safety issues, such as deficient water treatment and delivery, aging and deteriorating of the infrastructures, disinfection and antibiotic-resistant microbial populations, and transfer of virulence factors, are occurring worldwide, and cannot be circumscribed geographically [32].

Widespread urbanization, and "the rush from farm to city," the development of cities into "massive projects in the intensive monoculture of humans," and centralization of production and large-scale distributions have caused irreparable losses of traditions and know-how from rural areas, have increased the opportunities for foodborne disease transmissions, and have generated new challenges and new scenarios in food chains [160]. In agrarian societies, consumers personally observed the whole food chain "from harvest to consumption," but in modern long and complex food chains, consumers have no choice but to "rely on unseen third parties to scrutinize the safety and wholesomeness of perishable foods and to protect them against natural and man-made hazards" [197]. Traditional inspections from the times of backyard slaughter to recent times relied on the "visual identification of foodborne hazards" and focused on the contamination of water and food supplies with sewage or animal manure right before consumptions [164]. But "the vast majority of foodborne diseases occurring today are caused by microorganisms that cannot be detected" through such tangible signs [197]. And the sources of these contaminations tend to occur earlier "in the production process, rather than just before consumption" [164, 189].

Recent advances in science, technology, and medicine have modified patterns of food consumptions and have "brought to light unrecognized foodborne hazards." For example, nutritional dietetic requirements recommending more balanced diets and an increased consumption of raw fruits and vegetables [176] have caused many changes in "raw food production and harvest practices" [160], which have also opened the lid to new zoonotic pathogens, unknown just a few decades ago [176]. These new scenarios, combined with trends of vertically integrated ready-to-eat products; extended shelf lives; minimal food processing; packaging of semifinished marinates; popular and trendy marketing claims, such as low acid, low salt, and low sugar; as well as new fads, such as no preservatives or no additives added, etc., have further complicated the food safety scenarios. And advances in food science, technology, and medicine, compounded by demographic changes and by an increase of the "proportion of people with heightened susceptibility to severe foodborne infections," have generated unprecedented opportunities for new foodborne outbreaks to occur [160, 176, 198].

In a globalized world, pathogens, parasites, and toxins can travel faster than ever before along food chains [162], and international travel and immigration have resulted in the resurgences of unusual foodborne diseases in travelers and immigrant communities [176]. Furthermore, "for reasons that remain unclear," some emerging pathogens with identical genotypes have also appeared simultaneously in faraway locations, with no seemingly direct connections [164, 199]. But in spite "of the headlines that sometimes are triggered when an imported food is found to be the cause of a food-borne outbreaks," the vast majority of the foodborne illnesses are provoked by "domestically produced food." And even if the relative entities of domestic versus imported foods are usually not comparable, the stringent export specifications required for international commerce are not usually demanded in most countries to domestic and local producers as well [200]. Furthermore, inconsistencies between perceived and actual etiology of foodborne illness are common, because a significant proportion of outbreaks are underreported or caused by unspecified agents [187, 201, 202]. And in a "global village," "highly publicized outbreaks and severe cases" can "disproportionately affect perception of a pathogen incidence" of consumers, and professionals as well [203].

Implementation of food safety principles is achieved through some "spotty patchwork of standards and regulations," because major "differences in risk perception, surveillance, regulations, production systems" are common not only between but also within nations [189]. The Codex Alimentarius has been conceived as the comprehensive and "universal" approach for a unified and harmonized global food safety management worldwide. The Code, with its current set of 341 standards, guidelines, codes of practice, and technical recommendations, designed for the voluntary application by members [204], is used as reference in international food trade disputes by the World Trade Organization [205, 206]. The Code "General Principles of Food Hygiene" and the annex "HACCP" system and guidelines for its application are the roots of current hazard analysis and constitute the foundations of most national legislations [207]. Regrettably around the world however, "the adoption of HACCP procedures is both minimal and poorly policed, if at all," and many HACCP plans are "largely regarded as a 'necessary evil' and something that needs to be recognized rather than seriously applied" [208].

Frequently in HACCP plans, including some of the approved national guidelines, hazard analysis is conducted subjectively as far as the HACCP team remembers.

Determinations of Critical Control Points (CCPs) through a decision tree are often replaced by personal or official criteria, where anything important is automatically identified as a Critical Control Point. Critical Limits are also frequently extrapolated out of context and not sufficiently validated. Monitoring activities, corrective actions, verifications, and record keeping are at times poorly defined and executed [207, 209]. The US Food Safety Modernization Act is trying to address some of these issues through HARPC, which stands for "Hazard Analysis Risk-Based Preventative Controls." And even if it looks "a lot like HACCP on the surface," there are important differences worth noticing. HARPC requires the teams to be of "qualified individuals with training and experience" and does not differentiate "CCPs from other types of preventive controls," because "not all preventative controls have specific limits." The goal of the verification is not to ensure the compliance of the plan "but to validate the plan itself." And validation must come with sound "technical or scientific information that backs up the plan" [210].

Improved monitoring methodologies are needed to keep pace with these changes, because traditional "indicator organisms" such as coliforms and *E. coli* in potable water can be misleading, as these bacteria have been "found to grow in source waters even in absence of fecal contamination" and "pathogenic protozoa and viruses of fecal origin" have been found, where no "indicator bacteria" was detected [32]. The sale of raw drinking milk to consumers, which is not included in the scope of the Codex of hygienic practice for milk and milk products [211], is regulated through national legislations. And as a result, even within the same nation, the sale might be permitted, tolerated, regulated, or prohibited. Where it is regulated, consumers are usually advised to warm the milk to a temperature, e.g., 70 °C (158 °F) before consumption [212]. But where prohibited, people can resort to the "black market" or legal loopholes such as "cosmetic milk or bath milk" [213–223]. But prohibitions are difficult to enforce, and warning labels are not the most effective way "of conveying food safety messages" [189].

Traditions of raw milk and raw milk products are perceived differently around the world [224]. Likewise, people feel that free-range farm eggs are safer that "commercial eggs," and "studies have confirmed that caged hens in high-population density facilities produce *Salmonella*-contaminated eggs," but "free-range and cage-free hens are not exempt from this problem" [198]. The Codex hygienic practice for egg and egg products contemplates, where it is allowed, washing and sanitizing egg shells and coating them with food grade oil to prevent recontamination [225, 226]. Eggs should always be maintained at constant temperatures to avoid shrinkage of the albumen, which "can suck into the egg any bacteria that may be present on the outside of the shell" [198]. Refrigeration of eggs is mandatory in some countries and not in others, and delivery to consumer within a fixed time from the deposition (e.g., 21 days) is also enforced [227]. Where possible, eggs from flocks infected by *Salmonella* serovars of public health significance should be processed and marketed separately [228]. And as we learn more from food microbiomes, we may also change our criteria regarding indicators, spoilers, and deteriorating microorganisms. And may be, we may shed some light on the nutritional "French Paradox," whether it is caused by wines, by cheeses, or perhaps by microbes [229].

Recommendations

Food safety education should start early in life and should be based on local situations. The program of the World Health Organization (WHO) “Five Keys to Safer Food” is an essential part of a global strategy to decrease the burden of foodborne diseases and fills the need for clear, locally adapted tools, containing key global messages for food safety: (1) keep clean, (2) separate raw and cooked, (3) cook thoroughly, (4) maintain food at safe temperatures, and (5) use safe water and raw materials [230]. Furthermore, “Five Keys to Growing Safer Fruits and Vegetables” features essential core messages for safe vegetable and fruits: (1) practice good personal hygiene, (2) protect fields from animal fecal contaminations, (3) use treated fecal waste, (4) evaluate and manage risks from irrigation water, and (5) keep harvest and storage equipment clean and dry [231].

From an anthropological cross-cultural view, these locally adapted tools are well suited for people inside the “global village” but should also be extended to the ones outside the village, by providing, for instance, local and sustainable solutions, where there are no food thermometers or where people could not read them because they lack the skills or light. Albeit not specifically designed to train food handlers, the program “Clean Care is Safer Care” is a useful localized tool to convey principles of personal hygiene to food handlers as well. But this tool needs to be extended to other realities, for instance, where there is soap but no disinfectant or paper towels, buckets of cleaning water but no water tabs, or basic sanitation and plumbing does not provide hand-free water tabs [232, 233]. All humans should be entitled before birth to clean water, food security, improved sanitation facilities, adequate hygiene, professional health care, and education.

Education is the key factor in food safety, and consumers as the ultimate decision makers represent the weakest link. Consumers should be rendered more aware of the fact that the likelihood they “become ill due to an exposure to a foodborne pathogen” does not only depend on “bad bugs,” or on “the virulence characteristics of the pathogen,” or on the quantity or number of pathogens ingested, or by the food matrix characteristics. But it is the result of an integration of all these factors with their “general health” and particularly with their immune and microbiome status [182]. The octahedron concept of the elements of food safety presented in Chap. 3 is currently being developed into a mathematical model, where users through a series of questions regarding themselves and the food item they consume can potentially establish what risk level they are likely to be exposed.

Global food safety can only be achieved through the systematic elimination of existing social inequalities among humans, through the promotion of sound ecological principles and through the advancements of locally adapted food safety education programs.

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A. Zaccheo et al., *Food Hygiene and Applied Food Microbiology in an Anthropological Cross Cultural Perspective*, DOI 10.1007/978-3-319-44975-3