Foodborne Pathogens and Antibiotic Resistance
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Edited by Om V. Singh
The editor gratefully dedicates this book to Daisaku Ikeda, Uday V. Singh, and Indu Bala in appreciation for their encouragement.
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Preface

Food is an essential means for humans and other animals to acquire the necessary elements needed for survival. However, it is also a transport vehicle for foodborne pathogens, such as *Salmonella* and *Escherichia coli*, which can pose great threats to human health. Use of antibiotics (e.g., penicillin, kanomycin, streptomycin) has been enhanced in the human health system for multiple generations; however, selective pressure among bacteria allows the development for antibiotic resistance. Recent technological advances have opened the door to explore alternative solutions to antibiotics resistance that might prove useful toward food safety in food industry at large.

*Foodborne Pathogens and Antibiotic Resistance* features outstanding articles by expert scientists who shed light on broad-ranging areas of progress in the development of food safety interpreting antibiotic resistance. It bridges technological gaps, focusing on critical aspects of foodborne pathogen detection and mechanisms regulating antibiotic resistance that are relevant to human health and foodborne illnesses.

This ground-breaking guide:
- Introduces the microbial presence on variety of food items for human and animal consumption.
- Provides the detection strategies to screen and identify the variety of food pathogens.
- Reviews the literature on diversity of foodborne pathogens on varying food matrices.
- Provides microbial molecular mechanism of food spoilage.
- Discusses molecular mechanism of microorganisms acquiring antibiotic resistance in food.
- Discusses systems biology of foodborne pathogens in terms of detection and food spoilage.
- Discusses FDA’s regulations and Hazard Analysis and Critical Control Point (HACCP) toward challenges and possibilities of developing global food safety.

*Foodborne Pathogens and Antibiotic Resistance* is an immensely useful resource for graduate students and researchers in the food science, food microbiology, microbiology, and industrial biotechnology.
Food is the way that humans and other animals acquire the necessary elements needed for survival. However, it is also a transport vehicle for foodborne pathogens, such as *Salmonella* and *Escherichia coli*, which can pose great threats to human health. Raw food, ready-to-eat vegetables, dairy products, pork, beef, and poultry have been shown to harbor antibiotic-resistant pathogens, as well as multi-drug-resistant pathogens. Food spoilage can be a visual sign of pathogenic bacteria. Spoilage due to enzymatic oxidation and the reduction mechanisms of pathogenic bacteria can be prevented if appropriate measures are taken to preserve the food: heating, cold preservation, fermentation, moisture reduction, chemical preservation, ultra-high pressure, or irradiation.

In the United States, foods are regulated under the Federal Food, Drug, and Cosmetic Act (FD&C Act; 21 U.S.C. Sec 321). The classification of food products (“i. articles used for food or drink for man or other animals, ii. chewing gum, and iii. articles used for components of any other such article”) defines how rigorously the food or related product is regulated, or if the product is even legal to consume as a food. Food adulteration is defined in section 342 of the United States Code of Federal Regulations (CFR), title 21, chapter 9 (FD&C Act), subchapter IV (food). According to the FD&C Act, “food is [also] adulterated if it has been prepared, packed, or held under unsanitary conditions; may become contaminated with filth; or has been rendered injurious to human health.” To make sure food does not become adulterated and human illness does not occur, levels of microorganisms need to be kept low or nonexistent. In general, federal and state law prohibits the selling of adulterated foods.

Worldwide, significant efforts are being made to improve food safety with new policies and recommendations. Antibiotics (e.g., penicillin, kanomycin, and streptomycin) have been used in food safety and healthcare for multiple generations; however, selective pressure among bacteria has allowed the development of antibiotic resistance. Among these bacteria are foodborne pathogens, which have acquired antibiotic resistance genes through horizontal gene transfer and mobile genetic elements (e.g., transposons, plasmids). Due to the overusage of antibiotics in the agricultural system, antibiotic resistance genes have been transferred through agricultural waste, soil, meat, vegetables, and water systems. There are a variety of alternatives to antibiotics (i.e., phage therapy, bacterial vaccinations, probiotics, and prebiotics) that are undergoing evaluation, but are not as reliable due to many shortcomings such as cost-effectiveness, specificity, and likelihood of bacteria becoming resistant. Human society must learn from ongoing microbial outbreaks to deal better with emerging antibiotic resistance and multiple-drug resistance.

This book continues to bridge the gaps on technology and focuses on exploring the diversity of food pathogens in varying food matrices, in addition to the inescapable question of antibiotic resistance among foodborne pathogens and its impact on human society.
Micallel in Chapter 1 present the prevalence of common as well as under-researched bacterial pathogens and parasites along with the limitations of current detection techniques. They lay out a future direction toward improving detection techniques for under-researched foodborne pathogens and parasites in produce and animal products.

The connection between seafood and human health is undeniable; the National Marine Fisheries Services (NMFS) yearbook of fishery statistics for the United States for 2014 reported that the estimated U.S. per capita consumption of fish and shellfish was 14.6 pounds (edible meat) in 2014. The United States is the second largest consumer of seafood in the world, after China and before Japan. Bolivar et al. in Chapter 2 explain and characterize foodborne pathogens and spoilage bacteria in Mediterranean fish species and seafood products. The same chapter provides insights into European seafood regulations and microbiological standards for fishery and aquaculture products. Notably, the authors also present the global legislative requirements for controlling the safety and quality of fish and seafood products defined under Hazard Analysis and Critical Control Point (HACCP).

The *Pseudomonas* genus is one of the most diverse ecologically significant groups of known bacteria, and includes species that have been isolated worldwide in all types of environments. This genus contaminates foods from many sources and is able to utilize a wide range of materials, such as red meat, fish, milk and dairy, etc., as substrates for growth. In Chapter 3, Raposo et al. give an overview of the mechanistic views of food spoilage by *Pseudomonas* spp.

The technological implementation of pathogen detection is an undeniable aspect of food safety. The microorganism *Arcobacter* spp. is a food- and waterborne pathogen distributed throughout the food chain. In fact, consumption of *Arcobacter*-contaminated food or water is the most common cause of infection that poses a serious hazard to human health. Ferreira et al. in Chapter 4 explain the specifics of *Arcobacter* and human and animal infections associated with this genus. The chapter also describes unique “-omics” molecular approaches to detect *Arcobacter* in varying food matrices.

Vegetables are important for a healthy diet, as they are sources of phytosterols, dietary fiber, phytochemicals, minerals, and water-soluble vitamins, among other important compounds. Table olives are a traditional fermented vegetable with many centuries of history; they are a good source of antioxidants (hydroxytyrosol, oleuropein, methyloleuropein, etc.) and triterpenic acids. The global consumption of olive oil has almost doubled over the last 25 years, with a jump of 73%. Based on the importance of the health benefits from olives, Valero-Diaz et al. in Chapter 5 describe varieties of olives, microbial hazards, and their implications in the production of table olives.

Under stress, many microorganisms can temporarily change their genetic expression to form spores or endospores. Bacterial species can thrive under adverse environmental conditions such as food sterilization in this way, potentially causing foodborne diseases. Voundi et al. in Chapter 6 describe the phenomenon by which a vegetative cell becomes a spore and goes through the germination process under favorable conditions. The chapter also highlights the problem of controlling spore-forming bacteria in food.

Due to challenges in timely detection of foodborne pathogens, the Centers for Disease Control and Prevention (CDC) reports, a staggering number of people die annually due to foodborne diseases, even though the United States has the safest food supplies in the world. In Chapter 7, Law et al. examine rapid detection methods and their applications for four major foodborne bacterial pathogens, that is, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Escherichia coli*, and *Salmonella*, along with their advantages and limitations.
Salmonella sp. has been recognized as the main culprit in food poisoning around the world. Zadernowska and Chajecka-Wierzchowska in Chapter 8 describe alternative rapid detection methods for Salmonella in food.

Many different measures have been used to prevent food spoilage. Genetic engineering is one new measure. In Chapter 9, Carroll and Zhou describe the use of CRISPR (clustered, regularly interspaced, short palindromic repeats) in bacterial genome editing for food safety and industrial purposes. Biswas and Mandal in Chapter 10 propose other natural modes of food safety, such as the use of natural antimicrobials against meatborne pathogens for food safety.

Antibiotics have long been important for treating infectious diseases in human and animals. Their use in the food industry has risen rapidly in the past few decades, and the overuse of antibiotics has caused bacteria to develop a means of protection against them, that is, resistance genes. Cole and Singh in Chapter 11 discuss the apparent linkage of foodborne pathogens with antibiotic resistance, describing the biochemistry of food spoilage, food preservation techniques, and systems biology approaches as effective ways to detect foodborne pathogens. Additionally, the chapter discusses alternative strategies for targeting foodborne pathogens. In continuation, Nair et al. in Chapter 12 discuss common preservatives and disinfectants used in the food industry, their modes of action, bacterial responses to these antimicrobials, and resistance mechanisms.

Many foodborne pathogens cause infectious diseases. Chemotherapy is being considered as a prominent mode of treatment, but bacterial variants continually evolve resistance to clinically relevant antimicrobial chemotherapeutic agents, potentially confounding effective treatments, especially when the pathogens are multi-drug-resistant. In Chapter 13, Ranjana et al. discuss recent developments in key multidrug efflux pumps of the major facilitator superfamily from key foodborne bacterial pathogens.

As stated earlier, the foodborne pathogen Salmonella sp. is ubiquitous in agricultural, environmental, and human reservoirs, causing human gastrointestinal illness worldwide. The National Antimicrobial Resistance Monitoring System (NARMS) recognized the development of antibiotic-resistant Salmonella, which has been a public health concern for over 40 years and continues to persist in nontyphoidal Salmonella (CDC, 2015).6 In Chapter 14, Brunelle et al. explore the prevalence, evolution, and dissemination of antibiotic resistance in Salmonella.

The genus Staphylococcus is a broad group of microorganisms containing coagulase-positive staphylococci (CPS) and coagulase-negative staphylococci (CNS). Chakecka-Wierzchowska and Zadernowska in Chapter 15 describe antibiotic resistance in both CPS and CNS isolated from food.

Enterococcus spp. microorganisms have been used for decades in food fermentation and preservation. Organisms in this genus have also emerged as nosocomial- and community-acquired pathogens. Due to the nature of pathogenicity, the Enterococcus spp. are regarded as reservoirs of antimicrobial resistance genes and indicators of antibiotic resistance. In Chapter 16, Vangelis et al. describe antibiotic resistance in Enterococcus spp.

Seafood remains on the top tier of the global food market that has led to food mobility across continents, but also increases the chances of food contamination with a number of pathogenic microorganisms. Kumar et al. in Chapter 17 describe antibiotic resistance in seafood-borne pathogens.

Campylobacter spp. are part of the normal intestinal flora of wild and domestic animals, including birds, and the most frequently recognized bacterial cause of human gastroenteritis. Poultry and their meat products are the main source of Campylobacter spp. contamination that affects humans. In Chapter 18, Osaili and
Alaboudi describe occurrences of *Campylobacter* sp. and its antimicrobial resistance in animals and humans.

Advancements in technology have greatly improved the lifestyles of people living in developed countries, but developing nations are still struggling with the basic necessities. Foodborne diseases due to pathogenic organisms are one common issue that has not received much attention from local and international authorities. In Chapter 19, Adeyanju discusses the prevalence and antibiograms of foodborne pathogens in developing African countries. Globally, the misuse of antibiotics is another issue to be raised, as it promotes bacterial growth and resistance against multiple antibiotics. In Chapter 20, Skariyachan et al. discuss the evolution and prevalence of multi-drug resistance among foodborne pathogens.

This book, *Foodborne Pathogens and Antibiotic Resistance*, is a collection of outstanding articles elucidating several broad-ranging areas of progress and challenges related to foodborne pathogens. This book will contribute to research efforts in the scientific community and commercially significant work for corporate businesses, with the goal of establishing a long-term safe and sustainable food supply with minimum impact on the necessary elements needed for survival.

We hope readers will find these articles interesting and informative for their research pursuits. It has been my pleasure to put together this book with Wiley-Blackwell Press. I would like to thank all of the contributing authors for sharing their quality research and ideas with the scientific community through this book.

References


1.1 Introduction

The Centers for Disease Control and Prevention (CDC) estimates that financial losses from foodborne illnesses, including medical costs and losses in productivity, range from $500 million to $2.3 billion annually. More than 250 foodborne diseases are recognized and the major causative agents associated with foodborne illness are bacteria, virus, and parasites. *Salmonella*, *Campylobacter*, pathogenic *E. coli*, *Listeria*, *Shigella*, and *Vibrio* are the most common foodborne pathogens associated with meat and animal products (Mead, 2004; Hutchison et al., 2005). A decade ago, animal origin products used to cause known cases of foodborne disease, but now the whole scenario has been changed due to current health food habits in the United States. Green vegetables and fruits are highly recommended for a healthy life, and in the United States, consumption of produce has increased significantly in the last decade (Smith et al., 2013; Sivapalasingam et al., 2004; Scallan et al., 2011). Further, organic produce has been shown to be of superior nutritional value, compared to conventional produce (Lester and Saftner, 2011; Hallmann, 2012; Hallmann and Rembialkowska, 2012; Vinha et al., 2014). Many consumers opt for organic products due to their nutritional value, and because the produce is free of synthetic pesticides and antibiotic residues. Simultaneously, the CDC reported that the proportion of foodborne bacterial disease outbreaks associated with fruits and vegetables have increased significantly (Johnston et al., 2005; Berger et al., 2010; CDC, 2011; Gould et al., 2013). More specifically, the microbiological quality of organic versus conventional produce has only been compared for some produce types (Pagadala et al., 2015; Marine et al., 2015). According to CDC outbreak data, plant products, including fruits, vegetables, spices, and grains, are responsible for >51% (4,924,877 recorded cases) of foodborne illness in the United States (CDC, 2013). Among plant products, produce-only origin attributed to more than 45% (4,423,310) of recorded cases (CDC, 2013). A wide spectrum of pathogens has been documented in produce-associated outbreaks and a significant number of the infectious agents (>20%) that were responsible for the produce-borne infections are unknown (Scharfe, 2011; CDC, 2013). Further, these numbers do not represent the actual number of cases of foodborne infection in the United States, because sporadic cases remain largely unreported and/or undiagnosed.
On the other hand, animal food products including meat, egg, and milk are commonly known as a major contributor to zoonotic infections (Mead, 2004; Hutchison et al., 2005), as many of the zoonoses are commonly found in farm animal gut as normal flora. In addition, with commonly known bacterial foodborne pathogens, farm animals also harbor varieties of under-researched microbial pathogens, specifically, parasite and viruses. Food processing and packaging facilities struggle with limitations in resources, and often lack in the latest scientific information and techniques to detect the possible contaminants that exist in these type of food products. According to the diverse sources and causative agents of foodborne infection, the quality control practices in food processing, storage, and transportation facilities in the United States need to make further progress to meet the current demand in reducing/controling foodborne infections.

1.2 Common Bacterial Pathogens and Parasites Found in Produce and Animal Products

Most studies have investigated only major and mostly known foodborne pathogen prevalence, such as Salmonella, Campylobacter, pathogenic E. coli, Shigella, Vibrio, and Listeria (Berger et al., 2010; Kozak et al., 2013; Bolton et al., 2012; Cartwright et al., 2013; Johnston et al., 2005; Mukherjee et al., 2006; Yokoyama et al., 1998; Peralta et al., 1994; CDC, 2008; Scallan et al., 2011; Zhao et al., 2014). Salmonella enterica subspecies enterica serovar Typhimurium (S. Typhimurium) and serovar Enteritidis (S. Enteritidis) are the most common serovars, and can cause disease syndromes, such as gastroenteritis and systemic infections, in a wide range of animal species and humans (Yokoyama et al., 1998; Peralta et al., 1994). Major produce-associated serotypes include S. Javiana and S. Newport, with fruits and nuts most commonly associated with the former, whereas vine and stalk vegetables are associated with S. Newport (Painter et al., 2013). It has been reported that approximately 10–20% of the poultry meat at the retail level is positive for many different serotypes. The most frequently reported serotypes in layer flocks in 2002 were S. Enteritidis (57.7%), S. Typhimurium (9.6%), and S. Infantis (6.9%) (Mead, 2004).

Another major foodborne pathogen, Campylobacter jejuni, is a microaerophilic, spiral-shaped, Gram-negative bacterium and causes bacterial gastroenteritis worldwide. The CDC estimated that C. jejuni causes 2.4 million cases in the United States each year (CDC, 2008) and is the causative agent for 5–14% of all diarrheal diseases worldwide (CDC, 2008). Campylobacteriosis, gastroenteritis with C. jejuni, is characterized by the rapid onset of fever, abdominal cramps, and bloody diarrhea (Skirrow and Blaser, 1992). Sporadic cases are most common and are often associated with handling and consumption of undercooked poultry, as C. jejuni is part of the normal intestinal flora in chicken (Shane, 1992; Skirrow and Blaser, 1992; Deming et al., 1987; Tauxe, 1992; Biswas et al., 2007). The presence of C. jejuni in processed chicken carcasses offered for retail sale was reported to range from 7% to 32% during the winter months and from 87% to 97% during the summer months (Willis et al., 2000). Campylobacteriosis is less commonly attributed to fresh produce, although a major C. jejuni outbreak in Alaska associated with raw peas contaminated with bird feces demonstrated the risk of fresh crop contamination posed by wildlife (Gardner et al., 2011).

Pathogenic foodborne E. coli, specifically enterohemorrhagic E. coli (EHEC), enteropathogenic E. coli (EPEC), and enterotoxigenic E. coli (ETEC) are also involved in thousand of foodborne infections in the United States (CDC, 2014). Foodborne pathogenic E. coli O157:H7 is commonly discussed in the media in association with foodborne illness outbreaks, because of the severity of the disease. The major reservoir of this bacterial pathogen is cattle, and eating raw or undercooked ground beef or drinking unpasteurized beverages or dairy products are mostly
associated with the bacterial infections (CDC, 2014). Major produce-associated outbreaks have demonstrated the adaptability of these pathogens to the plant niche, especially leafy greens (Grant et al., 2008; CDC, 2006; Marder et al., 2014) and sprouts (Scheutz et al., 2011).

Listeria spp., including L. monocytogenes, are commonly found in soil, water, and decaying plant material (Weis and Seeliger, 1975; Linke et al., 2012). As such, there are many potential routes for contamination of foods with this organism. One characteristic that makes L. monocytogenes particularly difficult to control is its ability to grow in foods at refrigeration temperatures. Although L. monocytogenes has been known as a human pathogen since the early nineteenth century, it has only recently been recognized as a foodborne pathogen (Pradhan et al., 2009). Several large outbreaks of listeriosis were reported due to consumption of contaminated foods, specifically refrigerated, ready-to-eat foods, such as hot dogs and deli meats, unpasteurized milk and dairy products, and raw and undercooked meat, poultry and seafood, salad, and fruits (CDC, 2011). The largest outbreak associated with fruit was caused by contaminated cantaloupe in 2011, sickening 146 people and causing 30 fatalities and one miscarriage (CDC, 2011).

Seafood consumption in the United States has been associated with a number of foodborne bacterial infectious agents. Specifically, Vibrio parahaemolyticus has been associated with sporadic infections and outbreaks of gastroenteritis, whereas V. vulnificus infections occur almost exclusively as sporadic cases. Clinical symptoms most often associated with V. parahaemolyticus infection include watery diarrhea, abdominal cramps, nausea, and vomiting; wound infections and septicemia occur less commonly (Iwamoto et al., 2010, Daniels et al., 2000). V. vulnificus is particularly virulent, especially among patients with liver disease and iron storage disorders, which are at increased risk of invasive infection such as sepsis and bacteremia (Iwamoto et al., 2010; Levine and Griffin, 1993).

In addition to bacterial pathogens and viruses, the risk of contamination of animal and plant food products with parasites exists. Parasites remain understudied because of the complexity of methods of isolation and identification. Therefore, minimizing the risks and enhancing intervention strategies to prevent cross-contamination of organic and conventional animal products and produce with parasites is a priority. Prevalence of parasites in various food products is crucial for the development of effective control strategies against identified risk factors and management of foodborne infections with under-researched pathogens. The possibility of contamination of produce grown in organic or integrated crop-livestock farms with parasites such as Cryptosporidium parvum/hominis, Cyclospora cayetanensis, and Giardia duodenalis are potentially high (Putignan and Menichella, 2010; Pullin, 1987). Recent C. cayetanensis outbreaks have proved difficult to trace back and control, including ones associated with cilantro and other unidentified products (CDC, 2013; Nichols et al., 2015). Another parasite commonly found in various farm animals including pig and chicken is Toxoplasma gondii (Dubey and Hill, 2002). Toxoplasmosis caused by T. gondii is an emerging public health problem in individuals who are at high risk for foodborne illness—pregnant women, infants, older adults, and people with weakened immune systems. Animals raised in unconfined conditions are at higher risk of being contaminated at all levels from farm to retail (Guo et al., 2015).

1.3 Unusual Bacterial Pathogens and Parasites in Produce and Animal Products

The CDC has estimated that less than a fifth of estimated foodborne illnesses per year are attributed to a known agent, with over 38 million remaining unknown (CDC, 2011; Painter et al., 2013). Further, the prevalence of lesser-known or under-researched zoonotic pathogens and
their roles in cross-contamination of produce and etiology of human gastrointestinal infections have not been investigated in depth. This paucity of data is attributable to difficulties in identifying cases and the lack of reliable methods for detecting certain bacterial pathogens and parasites in animal and plant food products (Jolly and Lewis, 2005). Likely, under-researched zoonotic pathogens enter the food chain through direct contamination with fecal matter from farm animal to animal and plant food products, or indirectly via contaminated soil or water contaminated with fecal matter. Table 1.1 summarizes several unusual/under researched bacterial pathogens and parasites, and their sources caused outbreak or sporadic cases of foodborne infection.

In a study in the United States’ Upper Midwest region, Mukherjee et al. (2006) investigated the contamination level of organic produce with common zoonotic bacterial pathogens at pre- and post-harvest levels and concluded that some of the conventionally produced fruit and vegetables had significantly lower coliform counts than did semi-organic (uncertified) or organic produce. In another study in Canada, Kozak et al. (2013) found that in addition to bacterial pathogens, several parasites were also often associated with produce-borne infections. This may vary depending on commodity, but to our knowledge, no data appears to exist on on-farm cross-contamination for under-researched microbial pathogens at any production scale. However, due to the proximity of animal and crop cultivation areas on smaller farms, it is possible that risks of pathogen dissemination onto produce are higher in small- and medium-scale mixed or integrated crop-livestock farm environments. Vehicular and human traffic, prevailing winds, rain run-off, and wildlife could all contribute to dispersal of human pathogens from animal rearing and manure composting areas to pre-harvest produce production areas (Salaheen et al., 2015).

The common livestock grown in integrated crop-livestock farms are pig, goat, sheep, cattle, and poultry (Hoffman, 2010; Strawn et al., 2013). These livestock are known major reservoirs for zoonotic pathogens, including under-researched foodborne pathogens, the prevalence of *Staphylococcus aureus* and *Yersinia enterocolitica* in produce are quite high, and these pathogens are not studied yet at all in the United States. Integrated crop-livestock farm products such as fresh fruits and vegetables (spinach, carrots, lettuce, tomatoes, cucumber, apples, and strawberries) are high-risk foods with respect to contamination with these bacterial pathogens. In addition, the possibility of contamination of the produce grown in integrated crop-livestock farm with parasites such as *Cryptosporidium parvum/hominis* and *Giardia duodenalis* are potentially high and these parasites are mostly unknown because of the complex methods of isolation and identification. Therefore, minimizing the risks and intervention strategies of cross-contamination of organic or conventional produce with these under-researched bacterial pathogens and parasites are required. Such data are crucial for the development of effective control strategies against identified risk factors and management of foodborne infections with under-researched pathogens.

### 1.4 Farming Systems and Mixed (Integrated) Crop-Livestock Farming

In a European study, it was found that the level of contamination with foodborne pathogens was higher in produce samples cultivated under organic practices on integrated farms compared to those grown in produce-only farms in the absence of livestock (Bolton et al., 2012). Parasites such as *Giardia*, *Cryptosporidium*, and many bacterial pathogens including *Salmonella*, *E. coli O157:H7*, *Staphylococcus*, and *Yersinia*, could be introduced to integrated or mixed crop-livestock farms (MCLF) and its products at the pre-harvest level through contaminated water, dirt, insects, animal waste fertilizer, shared/commonly used instruments, and/or farm animals, birds, and wild animals (Natvig et al., 2002). It appears, however, that