Food Microbiology and Food Safety
Practical Approaches

Kumar Venkitanarayanan
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Steven C. Ricke *Editors*

Food Safety in Poultry Meat Production

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Practical Approaches

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Food Safety in Poultry Meat Production

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Preface

Poultry meat is an economical source of high-quality protein for human consumption. The United States leads the world in poultry meat production; the US poultry industry produced 55.6 billion pounds of broiler meat in 2016, with a monetary value of $28.7 billion. Poultry production at the global scale is projected to dominate across the different meat types and account for 45% of the total meat produced over the next 10 years. The peaking growth in broiler meat production over the past decades primarily stemmed from systematic genetic selection of poultry breeds targeting greater feed conversion efficiency, short production cycle compared to other food animals, and growth. Further, the use of antibiotics as growth promoters in poultry feed also contributed to increased body weight gain in birds.

The microbiological safety of poultry meat is essential for the economic viability of the poultry industry. *Salmonella* and *Campylobacter* are two major food-borne pathogens epidemiologically linked to the consumption of poultry products. Although the poultry industry implements several interventions to improve food safety, poultry-borne outbreaks linked to *Salmonella* and *Campylobacter* persist to occur, resulting in significant economic losses and adversely affecting public health. In addition, we continue to face the challenge posed by drug-resistant bacterial strains, including multidrug-resistant *Salmonella* Heidelberg and fluoroquinolone-resistant *Campylobacter jejuni* that have a significant impact on human health. Thus, sustained research to develop interventions to control these two pathogens and others, both at pre-harvest and post-harvest broiler meat production, are critical.

Although the growth-promoting efficacy of antibiotics in livestock and poultry has not decreased despite their prolonged use over decades, their continued use in animal agriculture especially at sub-therapeutic levels has received substantial scrutiny from the scientific community and regulatory agencies. This is due to the development of antibiotic resistance in food-borne bacteria and evidence linking antibiotic use in animal agriculture to potential resistance development. Thus, in light of consumer demand for a wholesome diet with enhanced food safety, the US Food and Drug Administration recently issued a directive, limiting the use of antibiotics in livestock and poultry production as growth promoters. This has triggered intensive research to identify effective alternate growth promoters to antibiotics in poultry

production. The steady growth of the US organic poultry sector has also fueled the need for growth promoters alternative to antibiotics. There is also a need to strengthen farm biosecurity measures and find new and efficacious antimicrobials that can counteract the challenge posed by drug-resistant pathogens.

The gastrointestinal tract (GIT) is the largest immune organ of the body, and GIT health in chicken is increasingly gaining research attention, since it is recognized as critical for bird welfare and productivity. This is because GIT health includes a complex unison of interrelated factors such as nutrient digestion, absorption, epithelial barrier function, endocrine regulation, gut microbiome, and mucosal immune responses. A thorough knowledge of how management and dietary factors, including antibiotics and non-antibiotic growth promoters which affect GIT health, would help in enhancing overall health and performance of chickens and poultry product safety.

Scientific advancements in microbiology, molecular biology, and immunology, coupled with access to next-generation "omics" disciplines such as genomics, transcriptomics, and metabolomics, have intensified our efforts for improving the microbiological safety of poultry by targeting virulence mechanisms of pathogens, developing new-generation vaccines, and enhancing gut health in chickens. Readers of this book are expected to gain a comprehensive understanding of current information on all aspects of food safety in poultry meat production. We sincerely thank our colleagues who worked hard to provide their input to the different chapters. It would not have been possible without their valuable contributions.

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Contents

Chapter 1
Salmonella in Poultry Meat Production

Divek V. T. Nair and Anup Kollanoor Johny

1.1 *Salmonella*: A Major Foodborne Pathogen in Poultry

Foodborne illness caused by various pathogens represents a major public health concern that results in significant loss to the U.S. economy (Marder et al. 2017; Scharff 2012). In 2016, the Centers for Disease Control and Prevention (CDC) Foodborne Diseases Active Surveillance Network (FoodNet) identified 24,029 infections, 5512 hospitalizations, and 98 deaths caused by pathogens such as *Campylobacter*, *Cryptosporidium*, *Cyclospora*, *Listeria*, *Salmonella*, Shiga toxin-producing *E. coli*, *Shigella*, *Vibrio*, and *Yersinia* (Marder et al. 2017). Among the bacterial pathogens associated with foodborne illness, non-typhoidal *Salmonella* (NTS) caused the second largest number of confirmed and culture-independent diagnostic test (CIDT)-positive infections (8172 cases) in the USA, second only to *Campylobacter* that caused 8547 illness cases (Marder et al. 2017).

Salmonella is a major foodborne pathogen implicated in outbreaks causing human illness for over a century (Bean and Griffin 1990; CDC 2000, 2013; Chalker and Blaser 1988). The organism is historically considered as the causative agent of the "meat poisoning" outbreak reported in Germany in 1888 and was first isolated by A. Gärtner, naming it as *Bacillus enteritidis*. In the USA, salmonellosis was designated as a notifiable disease in 1943, and since then, a steady increase in the reported incidence of *Salmonella* has been noted (Angulo and Swerdlow 1999; Tauxe et al. 1989). Since the mid-1980s, the pathogen gained tremendous importance due to its association with foodborne illnesses worldwide (Rodrigue et al. 1990; Tirado and Schmidt 2001). Currently, many serotypes of *Salmonella* are prevalent, and others are emerging as health threats to humans who contract the infection

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by consuming *Salmonella*-contaminated food products, the major animal-derived foods being poultry meat and eggs.

Salmonella is a Gram-negative, non-spore-forming, and motile bacillus belonging to the *Enterobacteriaceae* family. It is a facultative anaerobe that grows between 8 °C and 45 °C and at a pH range of 4–8. The pathogen is broadly classified into typhoidal and NTS based on host adaptability and infectious nature. The NTS has a wide range of vertebrate hosts, whereas host range of typhoidal *Salmonella* is limited to humans (Feasey et al. 2012; Winter et al. 2010).

Since chickens serve as natural hosts for many NTS serovars, the pathogens are frequently isolated from poultry and poultry products, with meat and shell eggs being the most commonly implicated vehicles in outbreaks. Most serovars of *Salmonella* colonize almost every part of the chicken intestinal tract, with highest predilection potential noticed in the paired blind sacs at the hind end of the tract called the ceca. Once colonized, the pathogen can be excreted through the feces without chickens showing any obvious clinical signs of infection. This eventually leads to the horizontal transmission of infection to other healthy birds and flocks, contamination of carcasses during slaughter, contamination of eggs with feces, and the retrograde transmission of infection via the transovarian route by major serovars such as *S*. Enteritidis and *S*. Heidelberg (Gantois et al. 2008; De Reu et al. 2006).

Although zoonotic in nature, NTS often causes self-limiting gastroenteritis in healthy humans. However, the infection process is more severe in immunocompromised individuals, children, and older adults, and the infectious dose can be low. The incubation period of the disease typically ranges 12–72 h with the illness lasting for 2–7 days. Patients usually recover within a week without any antibiotic treatment except in cases of severe diarrhea, where intravenous fluid therapy is warranted (Feasey et al. 2012). However, the severe illness caused by antibiotic-resistant strains of *Salmonella* may result in longer periods of stay in the hospital (Lee et al. 1994). The infection often proceeds to bacteremia and invasive form in immunocompromised individuals (Antunes et al. 2016; Chen et al. 2013). The fecal excretion of the pathogen from infected humans leads to the transmission of the pathogen among different vertebrate hosts (Dhanoa and Fatt 2009).

1.2 *Salmonella* in Poultry Production

1.2.1 S. *Pullorum and* S. *Gallinarum*

Diarrheal diseases have been a serious problem in poultry rearing/production systems that resulted in significant economic loss to the producers/industry, historically. *Salmonella* serovars such as *S.* Gallinarum and *S.* Pullorum were commonly isolated from poultry intestinal contents, droppings, and internal organs ever since poultry rearing was considered a financial enterprise (Stafseth and Mallmann

1928). The industry was aware of the importance of hygienic practices in poultry production to avoid diarrheal diseases from their valuable flocks. During that time, the major focus of the poultry sector was selection of superior breeds for improved egg production. Poor hatchability and smaller eggs were significant concerns, and the market trend was more toward producing eggs with superior hatchability. To aid this process, selective breeding and progeny testing were made common practices.

Although methods such as sanitation, immunization, and elimination of carriers and birds that showed signs of disease were practiced to control fowl pox and pullorum disease in hopes of saving the production strains (Hutt 1938), numerous outbreaks of *S.* Pullorum were reported in poultry in the early 1900s, and the carrier status of chicken for pullorum disease had been established. *S.* Pullorum emerged as a significant pathogen in poultry production. The egg-borne transmission of the disease was reported in 1909, and the septicemic nature of the pathogen was first reported in 1913. The young birds were mainly susceptible to *S.* Pullorum, and the disease was known as "fatal septicemia of young chicks" or "bacillary white diarrhea" or "pullorum disease" (Bullis 1977; Tittsler 1930). The bacterium was isolated from the liver, heart, lungs, and ovaries. A severe economic loss was reported due to the loss of egg production and mortality. The serum agglutination test and pullorum test were commonly employed to detect the disease in the flock (Tittsler 1930).

Commercial hatcheries became the source of infection, and the use of disinfectants was practiced in hatcheries. The dedicated incubators and use of formaldehyde for fumigation of eggs to control *S.* Pullorum became a common practice (Bullis 1977; Bushnell and Payne 1932). Responding to the situation, the National Poultry Improvement Plan (NPIP) was introduced in the USA in 1935 to control the pullorum disease. As a part of the NPIP, screening tests such as whole blood tests, tube agglutination tests, and rapid serum tests were used to detect *S.* Pullorum in the poultry flocks to eradicate and limit the disease. Based on the test results, the flocks were categorized into pullorum-tested flocks, pullorum-passed flocks, and pullorum-cleaned flocks (Bullis 1977).

However, *S.* Gallinarum caused fowl typhoid in adult chickens and was recognized as early as 1888. The tests that were used to screen pullorum disease were also used to detect *S.* Gallinarum. With the introduction of NPIP, the establishment of pullorum-free flocks also resulted in reduced incidence of fowl typhoid. In addition, the breeds such as White Leghorn were inherently resistant to these diseases, and the rearing of breeds resistant to infection became a common mitigation practice. Later, in 1954, screening of *S.* Gallinarum was also included as a part of NPIP. Moreover, antibiotics were used in poultry production to control these pathogens, resulting in tremendous improvement (Bullis 1977). Pullorum disease and fowl typhoid have been currently eradicated from the commercial flocks of developed countries such as the USA and Canada (Shivaprasad 2000). Although non-zoonotic, these pathogens still cause major economic problems in developing countries since they are highly adapted to poultry (Barrow and Freitas Neto 2011).

1.2.2 *Non-typhoidal* Salmonella

Though the eradication of *S.* Pullorum and *S*. Gallinarum could be achieved, the emergence of NTS resulted in significant safety concerns over the production of poultry meat and eggs for human consumption. The NTS *Salmonella* serovars caused 28% illness associated with foodborne outbreaks during 1973–1987 (Bean and Griffin 1990). On a later time-frame, 40% *Salmonella*-associated foodborne illness were reported in the USA from 1993–1998 (CDC 2000). The proportion of foodborne salmonellosis by poultry meat and eggs increased significantly from 1993 to 1998 compared to that occurred in the preceding decade. In the following decade (1998–2008), NTS *Salmonella* contributed 18% of the total illness associated with foodborne outbreaks in the USA (CDC 2013), underscoring a constant presence of NTS as the etiological agent in those outbreaks. *Salmonella* remains a major foodborne bacterial pathogen in the USA over a period of 50 or more years (Bean and Griffin 1990; CDC 2000, 2013; Chalker and Blaser 1988).

Two major epidemiological events that occurred in relation to the *Salmonella* serovars in the previous century were the emergence of *S*. Enteritidis as a major pathogen in poultry and the emergence of antibiotic-resistant strains of *Salmonella* (Rabsch et al. 2001). Although the poultry-adapted serovars of *Salmonella* such as *S.* Pullorum and *S*. Gallinarum were eradicated from commercial flocks in the USA by 1950, this successful event, however, created an environmental niche to be occupied by *S*. Enteritidis which was abundant in the rodent population. Since *S*. Gallinarum possessed cross-immunity against *S*. Enteritidis infection, it is reasonable to believe that the eradication of one resulted in the emergence of the other. In addition, higher bird density and vertical integration of poultry production system also facilitated the transmission of *S*. Enteritidis among poultry flocks (Foley et al. 2008, 2011).

1.2.3 S. *Enteritidis: A Major Serovar*

S. Enteritidis is the most genetically homogenous serotype of all *Salmonella* (Porwollik et al. 2005). Although limited in genomic diversity, the field isolates of the serotype vary in their capabilities to form biofilms, growth characteristics, production of high molecular mass lipopolysaccharides, and survival within the egg albumen (Clavijo et al. 2006; Jain and Chen 2007; Yim et al. 2010). In chickens, the pathogen varies in its virulence potential to cause mortality or to colonize the intestinal tract and invade the spleen and liver (Gast and Benson 1995, 1996). On-farm investigations indicate that once chickens are exposed to the pathogen, the entire flock can become colonized rapidly (Berrang et al. 2009; Foley et al. 2008). This could be attributed to the ability of the pathogen to proliferate in the gastrointestinal tract of chicken (Poppe 2000) and the multitude of sources in farms contributing to pathogen spread in birds.

S. Enteritidis is invasive in both young and adult chickens (Shah et al. 2011). Young chickens develop systemic disease with varying degrees of mortality (Duchet-Suchaux et al. 1995; Velge et al. 2005). The affected chicks may show all or some signs such as anorexia, depression, ruffled feathers, huddling together in groups, reluctance to move, drowsiness, dehydration, white diarrhea, stained and pasted vents, and stunted growth (McIlroy et al. 1989). However, adult chickens, once colonized with the pathogen, may remain as asymptomatic carriers, shedding the pathogen to the environment continuously or intermittently (Golden et al. 2008; Velge et al. 2005). Chickens infected with high doses of *S.* Enteritidis can subsequently develop clinical salmonellosis with high mortality, whereas infection with low doses will result in clinically healthy carrier birds (Desmidt et al. 1997; Gast and Benson 1995; Van Immerseel et al. 2004a, b). Currently, improvement in the vaccination strategies and the development of targeted interventions to control *S.* Enteritidis in/on eggs and meat have tremendously improved the situation. However, the emergence of other NTS serovars, such as *S.* Heidelberg, *S.* Oranienburg, *S.* Infantis, *S.* Hadar, *S.* Kentucky, and others, have raised serious concerns for the industry (Dutil et al. 2010; Foley et al. 2011; Wong et al. 2014; CDC, 2016; Hindermann et al., 2017).

1.2.4 Antibiotic-Resistant Salmonella

The development of antibiotic resistance in NTS serovars, including the most prevalent serovars such as *S.* Heidelberg and *S.* Kentucky, is an increasing concern for the U.S. poultry industry (Dutil et al. 2010; Foley et al. 2011; White et al. 2001). For example, the outbreak isolates of *S.* Heidelberg in the recent foodborne outbreaks were resistant to many clinically relevant drugs such as streptomycin, ampicillin, gentamicin, tetracycline, sulfamethoxazole, chloramphenicol, and trimethoprim-sulfamethoxazole. In addition, the isolates were resistant to the drug of choice to treat human salmonellosis—ceftriaxone (Medeiros et al. 2011; Foley et al. 2011; Hoffmann et al. 2014). Ceftriaxone-resistant *S.* Heidelberg was isolated from the retail meat sold in the USA (White et al. 2001) and Canada (Dutil et al. 2010). The resistant genes are encoded on plasmids in *S.* Heidelberg. *S.* Kentucky also possesses plasmids that encode genes for antibiotic resistance, resistance to disinfectants, iron acquisition, and bacteriocin production that enhance the survival of the pathogen in poultry flocks (Han et al. 2012).

Isolation of antibiotic-resistant strains of the *Salmonella* is not restricted to the U.S. poultry market. Jørgensen et al. (2002) reported that 70% of *Salmonella* isolated from 241 whole carcasses collected from retail stores in England were resistant to least one antibiotic, and 46% were resistant to more than one antibiotic. In a Portugal study, Antunes et al. (2003) detected 10 different serotypes of *Salmonella* from 60% of chicken samples, of which 50% were resistant to nalidixic acid and enrofloxacin. In a U.S. study, Cui et al. (2005) reported that all *S.* Typhimurium isolates obtained from retail chicken were resistant to more than five antimicrobials,

whereas those isolated from organic chicken were resistant to more than 17 antimicrobials. Out of the 569 samples positive for *Salmonella* (N = 4745), Roy et al. (2002) reported 92 samples collected from various environmental sources had isolates having resistance to erythromycin, lincomycin, and penicillin antibiotics, whereas all were susceptible to sarafloxacin and ceftiofur. In a different study, Parveen et al. (2007) found high levels of *Salmonella* from pre- and post-chilled poultry carcasses and water samples collected at the entrance of the chiller. Among the serovars isolated, 79.8% were resistant to at least one antibiotic, whereas 53.4% were resistant to more than one antibiotic, including tetracycline, ampicillin, amoxicillin-clavulanic acid, ceftiofur, streptomycin, and sulfisoxazole.

1.2.5 Salmonella *Serotypes in Poultry Meat Products*

It is well evidenced by the literature that poultry meat plays a major role in causing *Salmonella*-associated foodborne outbreaks since the 1950s (Bean and Griffin 1990; CDC 2000, 2013; Chalker and Blaser 1988). Poultry meat is the cheapest source of protein, and a large majority of U.S. population likes to have it in their diet (NCC 2017). Since poultry are the natural reservoirs of *Salmonella*, unhygienic processing and abused storage conditions of poultry meat can contribute to the incidence of salmonellosis in humans (CDC 2013).

Poultry meat, including the whole carcass, cut-up parts, and processed meats, are significant sources of several *Salmonella* serotypes that can cause disease in humans. In an early Canadian study, *Salmonella* was detected from 73.7% turkey carcasses and 38.2% chicken carcasses (Lammerding et al. 1988). Later, Logue et al. (2003) studied the incidence of *Salmonella* in two turkey processing plants in the Midwestern USA. Surface swabs were collected from poultry carcasses pre-chill and post-chill. Samples were also collected from the chill water. The overall incidence of *Salmonella* was found to be 16.7% after enrichment, and more positive samples were observed in pre-chill than post-chill. Major serotypes recovered were *S.* Senftenberg, *S.* Agona, *S.* Heidelberg, and *S.* Hadar. Jørgensen et al. (2002) studied the prevalence of *Salmonella* in 241 whole raw chicken samples purchased from retail shops in the UK at two different winter seasons of 1998/1999 and 1999/2000. The study found that *Salmonella* were present in 25% of the chicken samples. Among these, 19% of *Salmonella* was detected from both inside and outside of the chicken packages. The predominant serotypes detected were *S.* Indiana, *S.* Enteritidis, and *S.* Hadar (Jørgensen et al. 2002). Roy et al. (2002) detected *Salmonella* in 569 samples (11.99%) among 4745 samples collected from poultry liver and yolk sac, chicken ground meat, rinse water from spent hens and broilers, hatchery fluff, and drag samples from poultry environment during 1999/2000 in the Pacific Northwest. Out of the 97 positive samples serotyped, *S.* Heidelberg (25.77%), *S.* Kentucky (21.64%), *S.* Montevideo (11.34%), *S.* Hadar (5.15%), and *S.* Enteritidis (5.15%) were the major serotypes isolated. Likewise, the incidence of *Salmonella* in several poultry products obtained from a local butcher shop in Belgium revealed

that 60% of the samples were contaminated with *Salmonella* consisting of ten different serotypes. The most prominent serotypes isolated in the study were *S.* Enteritidis and *S.* Hadar (Antunes et al. 2003). In a study conducted in Spain to isolate *Salmonella* from 198 samples of chicken meat for sale in retail outlets, it was reported that the pathogen was isolated from 35.83% of the samples where the predominant serovars were *S.* Enteritidis (47.88%), *S.* Hadar (25.35%), and serotype 4, 12: b:-(II) (19.71%) (Domınguez et al. 2002). In yet another study conducted in Maryland, USA, Cui et al. (2005) reported 61% of organic and 44% of conventional chickens were contaminated with *Salmonella*. Between the years 2002 and 2006, *Salmonella* was isolated from 59.7% ground turkey, 36.9% chicken breast, and 3.4% pork chops among retail meat outlets in the USA (Zhao et al. 2008).

Frozen chicken nuggets, strips, and eggs were the main poultry foods implicated in the causation of human *S.* Heidelberg infections in Canada (Currie et al. 2005). Bohaychuk et al. (2006) detected *Salmonella* in 30% of raw chicken legs and meat and poultry products collected from a retail market in Alberta, Canada. In a Portugal study, Antunes et al. (2003) found *Salmonella* in 60 samples of poultry products obtained from local shops and canteens and detected ten different serotypes of *Salmonella* in 60% of samples and identified *S.* Enteritidis and *S.* Hadar as more prevalent. Jackson et al. (2013) studied the link between different *Salmonella* serotypes and various foods, including poultry, by analyzing outbreaks that occurred between 1998 and 2008. The study found that eggs and poultry meat were vehicles in more than 80% cases of *Salmonella* outbreaks caused by *S.* Enteritidis, *S.* Heidelberg, and *S.* Hadar. In another epidemiological study, Chittick et al. (2006) analyzed the national foodborne outbreak data from 1973 to 2001 and found that among 6633 outbreaks of known etiology, 184 (3%) were contributed by *S.* Heidelberg. Among these, 3 outbreaks were due to egg consumption, 17 cases were related to consumption of foods prepared using eggs, 25 cases were related to poultry, and 8 cases were due to consumption of food containing both poultry and eggs.

Foley et al. (2008) had observed that serovars *S.* Senftenberg and *S.* Hadar have become more prevalent in poultry, compared to *S.* Enteritidis, and *S.* Typhimurium. *S.* Heidelberg was reported to be more isolated from clinical cases and suggested to be more virulent than other serovars. The study concluded that among the top ten serovars of *Salmonella* associated with human infections, the majority were from swine and poultry, including *S.* Heidelberg. In a different study, Parveen et al. (2007) reported high *Salmonella* contamination in processed poultry products. In this study, 480 pre-chill and post-chill poultry carcasses and the chill water from entry and exit point were enriched and analyzed using an automated BAX system and culture methods to detect *Salmonella*. Approximately, 88.4% of pre-chill and 84.1% post-chill carcasses were found to be positive for the pathogen. In addition, 92% of the samples collected from entry points were found to be positive for *Salmonella*, whereas none were identified at the exit point. The predominant serotypes isolated were *S.* Kentucky (59.5%) and *S.* Typhimurium (17.8%) (Parveen et al. 2007). In yet another study, Lestari et al. (2009) studied the prevalence of *Salmonella* isolated from 141 conventionally raised and 53 organically raised chicken carcasses from 27 retail stores located in Baton Rouge, Louisiana. Recovery rates were similar.

Twenty-two percent of the conventionally raised chicken was found to be positive for *Salmonella*, whereas 20.8% organic chicken was found to be positive for *Salmonella*. Out of the eight serotypes isolated, predominant ones were *S.* Kentucky, *S.* Hadar, and *S.* Enteritidis (Lestari et al. 2009).

1.3 *Salmonella* in Vertically Integrated Production Systems

1.3.1 Breeders

Salmonella has multiple routes of entry in a poultry production system. Once the pathogen is introduced in poultry, the infected birds act as a constant source of infection through horizontal and vertical transmission of the pathogen in large poultry grow-out houses. *Salmonella* colonizes the reproductive organs such as ovary and oviduct, and during egg formation, the pathogen may enter internal contents such as the vitelline membrane and albumen (Gast et al. 2004, 2007; Heyndrickx et al. 2002). Subsequently, the chicks hatching from the contaminated eggs will serve as a source of infection to the flock. This is the common process involved in vertical transmission (Cason et al. 1994; Cox et al. 2000; Gast 1994). Therefore, the breeder stocks harboring *Salmonella* in the vertically integrated system have an imperative role in the prevalence and persistence of *Salmonella* in broiler meat production.

The constant presence of *Salmonella* in the poultry houses is mainly due to the vertical transmission of the pathogen from breeder flocks and horizontal transmission occurring in the housing facilities. *Salmonella* testing conducted in processing facilities of seven consecutive flocks of two vertically integrated broiler production systems in Georgia revealed a high prevalence *Salmonella* serovars such as *S.* Typhimurium, *S.* Montevideo, *S.* Kentucky, and *S.* Enteritidis. In addition, the carcass isolates of *S.* Enteritidis and *S.* Typhimurium showed indistinguishable PFGE patterns with the serovars isolated from the breeder flocks indicating the likelihood of *Salmonella* originating from the breeder flocks, subsequently contaminating the carcasses (Liljebjelke et al. 2005). Another retrospective study conducted by Crespo et al. (2004) also reported the continuum of *S*. Arizona from breeder flocks to eggs and meat.

1.3.2 Hatchery

In a vertically integrated broiler production system, hatcheries could be reservoirs of the pathogen, and the serovars of *Salmonella* present in processing environment are often traced back to hatcheries. Hatcheries harboring *Salmonella* could contaminate the eggs and eventually lead to the colonization in chicks (Bailey et al. 1994). *Salmonella* serovars can survive as an endemic population in hatcheries and can act

as a source of infection to the subsequent flocks (Bailey et al. 2002). *Salmonella* colonization in day-old chicks is of critical importance since the chicks are susceptible to the low infectious dose of *Salmonella.* In addition, less microbial diversity and an unstable gut microbiome will make the flocks susceptible to *Salmonella* (Oakley et al. 2014).

1.3.3 Farmed and Wild Animals, Rodents, and Other Vectors

Salmonella has wide host range and is distributed all over the environment. Domestic animals such as cattle, small ruminants, and pigs harbor NTS and act as a source of infection, especially in organic production or free-range settings (Davies and Wray 1996; Hoelzer et al. 2011). Wild birds such as raptors, vultures, crows, and gulls also serve as potential carriers of *Salmonella.* In addition, domestic pigeons, passerines, colonial water birds, finches, and house sparrows carry *Salmonella* in their intestines (Tizard 2004). *Salmonella* has been isolated from a wide variety of wild animals including squirrels, raccoons, foxes, mink, tigers, wild boars, rhinoceroses, seals, hedgehogs, and white-tailed deers. The transmission of *Salmonella* happens when infringement of wild and captive animals occurs (Hoelzer et al. 2011).

The carrier status of rodents for *Salmonella* serovars such as *S.* Typhimurium and *S.* Enteritidis often warrants pest control programs in poultry farms. It could be the direct transmission of the pathogen from the birds to the pests or vice versa (Wales et al. 2007). The rodents amplify the pathogen load in the environment and transmit those to the food animals, especially in the organic production system. Then the pathogen constantly circulates in the food chain (Meerburg and Kijlstra 2007). *Salmonella* prevalence in the farm premises due to rodents was estimated at 5.2% (Skov et al. 2008). Studies also revealed the genotypic and serological similarity between samples isolated from rodents and chicks (Liebana et al. 2003).

1.3.4 Human Traffic and Related Activities

Movement of people in and out of the farms is a major *Salmonella* introduction process in a poultry farm. *Salmonella* can be introduced into the farm through cages, feeders, drinkers, clothes, and boots (Wales et al. 2007). The movement of employees between different farms and contact with different species of animals are also potential threats to the safety. Therefore, proper physical barriers, disinfection procedures, dedicated clothes, and boots could be useful to reduce the introduction of the pathogens into the flock (Newell and Fearnley 2003).

The crates used for transportation of birds to the farms and processing plants carry *Salmonella. Salmonella* survives on crates even after washing them using quaternary ammonium compounds with an exposure time of 10 or 20 s. The flocks that were previously *Salmonella*-negative became positive from the contamination of the crates

(Slader et al. 2002). Therefore, the movement of portable equipment, including the transport crates could be an immediate source of *Salmonella* infection to the processing facility or poultry farms (Heyndrickx et al. 2002; Slader et al. 2002).

1.3.5 Feed, Litter, and Water

Contaminated feed is one of the main sources for contraction of *Salmonella* infection by poultry. Most of the time, the traditional techniques would not allow the recovery of a low level of *Salmonella* from the feed, although *Salmonella* numbers as low as ten cells can colonize in day-old chicks (Maciorowski et al. 2006; Park et al. 2011). Also, less than one *Salmonella* per gram feed is sufficient to cause colonization in 1- to 7-day-old chicks (Schleifer et al. 1984).

Salmonella survives in poultry feed in a strain-dependent manner. Most of the virulence genes are downregulated during its survival in a low water activity environment such as poultry feed (Andino et al. 2014). *Salmonella* serovars such as *S.* Typhimurium can persist in feed for months and act as a source of infection to the chicks or adult chickens. *S.* Typhimurium survives in feed for 40 days, 16 months, or 18 months at 38 °C, 25 °C, and 11 °C, respectively (Williams and Benson 1978). Therefore, hurdle technologies and intervention strategies are recommended during feed manufacturing, transportation, and storage (Maciorowski et al. 2004)

Salmonella persists in poultry litter and acts as a major source for intestinal colonization by the pathogen in chicks (Fanelli et al. 1970). Similar to the survivability in feed, serovars such as *S.* Typhimurium survives in the litter for months and acts as a source of infection to the chicks or broiler chickens. *S.* Typhimurium survives in the litter for 13 days at 38 °C and 18 months at 25 °C or 11 °C (Williams and Benson 1978).

Poultry drinking water can be contaminated with feed, litter, droppings, or dust carrying *Salmonella.* The residual organic contamination reduces the free available chlorine (FAC) in the water and changes the pH of the water which in turn reduces the efficacy of chlorination (Poppe et al. 1986). *Salmonella* at a level of 4–5 log CFU/ml has been recovered from the poultry drinking water. The main source of *Salmonella* contamination to the poultry drinking water is from the *Salmonella* attached to the trough drinkers and plastic bell drinkers (Renwick et al. 1992). Nipple drinkers are less likely to be contaminated with *Salmonella* because of their closed nature (Poppe et al. 1986). *Salmonella* forms biofilms in pipes and drinkers and acts as a persistent source of infection to the poultry (Poppe et al. 1986).

1.3.6 Aerosols

Salmonella survives in the aerosols, dust particles, and droplets. *Salmonella* persists in the dust particles for years and serves as a constant source for pathogen colonization (Davies and Wray 1996). The pathogen is often found in air inlets or fans and can be a recontamination source (Higgins et al. 1982). Studies conducted in a

controlled environment by regulating the air flow of the cabinet between challenged and non-challenged birds revealed that *Salmonella* could be transmitted from infected to non-infected birds via aerosols. Also, 33% of the non-challenged birds became infected with *S.* Enteritidis. Moreover, *S.* Enteritidis was isolated from the feathers of 77% non-challenged birds (Gast et al. 1998).

Aerosolizing of *S.* Enteritidis causes systemic infections through nasal and conjunctival routes (Baskerville et al. 1992; Humphrey et al. 1992) and elicits varying degrees of immune response in a dose-dependent manner. A low infectious dose of 10^3 CFU *S.* Enteritidis can cause lung infection and systemic infection in the liver, spleen, kidney, ovary, and oviduct in 2-day-old chicks. Also, the pathogen can be excreted through feces for 28 days (Cooper et al. 1996).

Currently different antibacterial interventions are practiced at the farm level to control NTS serovars. The interventions include prebiotics, probiotics, organic acids, short-chain fatty acids, vaccines, bacteriophages, and essential oils and are being supplemented through feed or drinking water (Atterbury et al. 2007; Callaway et al. 2008; Donalson et al. 2007, 2008; Higgins et al. 2008; Van Immerseel et al. 2006; Kollanoor Johny et al. 2009, 2012; Nair et al. 2016; Patterson and Burkholder 2003; Tellez et al. 2012; Zhang-Barber et al. 1999). These interventions will be explained in detail in a following chapter.

1.3.7 Processing Environment

Salmonella-colonized flocks excrete the pathogen through the feces that transmit the infection to the other birds in the flock, contaminating the poultry farm. The sharing of the common equipment also causes the introduction of the pathogen to the processing facility (Heyndrickx et al. 2002). Among the different stages of poultry processing, scalding, picking, evisceration, and chilling reduce the total microbial load on the carcass. In addition, cross contamination of carcasses is possible during these stages, if a single carcass is contaminated with *Salmonella* (Heyndrickx et al. 2002). Therefore, these are considered as critical operations in poultry processing in terms of reducing the prevalence of *Salmonella* on poultry carcasses (Svobodová et al. 2012). However, other steps of poultry processing are also important. For example, inappropriate stunning causes wing flapping and quivering which lead to soiling of the carcass with feces and transfer of *Salmonella* from inside to the outside of the body (Gregory 2005). Therefore, poultry processing is considered as a complicated and delicate procedure where a breach in the hygiene and sanitation affects public health that ultimately leads to billions worth product recalls in the industry.

1.3.7.1 Scalding

Scalding is the process in which broiler carcass is immersed at 59–64 °C for 30–75 s (hard scald) or 51–54 °C for 90–120 s (soft scald) to loosen up the skin for facilitating further picking (FSIS 2015). This is the first step in poultry processing where

the carcasses are immersed in water, and there is a high possibility of cross contamination with pathogens, including *Salmonella* (Carrasco et al. 2012; Russell 2008). A study conducted by Nde et al. (2007) revealed that *Salmonella* survived the scalding process, and the same isolates were identified before and after defeathering and from the rubber fingers of the defeathering equipment. *Salmonella* can be attached to the skin during the scalding process, evades the action of common antimicrobial agents, and acts as a source of infection in the subsequent stages of processing (Kim et al. 1996; Lillard 1990; Nchez et al. 2002; Yang et al. 2001). Also, a higher concentration of antimicrobial agent is necessary to kill *Salmonella* if it is attached to the skin surfaces (Yang et al. 2001).

1.3.7.2 Defeathering

Defeathering is another step in poultry processing where the possibility of cross contamination is high if contaminated water is used along with improper disinfection of rubber picking fingers (Nde et al. 2007). *Salmonella* that are attached to the skin on a carcass cross-contaminates other carcasses. A high prevalence of 47% and 63% *Salmonella* before and after defeathering, respectively, was noticed in this study (Nde et al. 2007). Other studies also reported significantly high *Salmonella*-positive carcasses after defeathering (71%) compared to that of pre-defeathering (21%) in the conventional defeathering method (Clouser et al. 1995a, b). Rubber fingers/picking fingers can cause peristaltic movements which also lead to the expulsion of feces (Berrang et al. 2001). Since the picking fingers are not changed between the carcasses, there is a high likelihood of carcass cross contamination (Nde et al. 2007). Therefore, sanitation using appropriate disinfectants is recommended during the defeathering process.

1.3.7.3 Evisceration

Evisceration is a critical step in poultry processing where an effective application of antimicrobial agents is recommended to prevent contamination of carcasses with intestinal contents. A faulty evisceration can lead to contamination of carcasses with fecal material and intestinal contents. Therefore, proper feed withdrawal before slaughtering, antimicrobial rinses such as chlorine, proper maintenance of evisceration machinery, and removal of ceca and crop without tear are recommended (FSIS 2015).

1.3.7.4 Chilling

Poultry carcasses are immersed in cold water during the chilling process to reduce the carcass temperature to 40 °F (4.4 °C) or below within 4–8 h of slaughtering to prevent the growth of pathogenic bacteria (FSIS 2014). The carcasses leaving the chillers often carry *Salmonella* (Lillard 1990; Nchez et al. 2002). Under natural

conditions, the carcasses exiting the chillers contains 1–30 CFU *Salmonella* per carcass (Waldroup 1996). The possibility of cross contamination is high in chillers compared to other steps in processing. Lillard (1990) showed 37% incidence of *Salmonella* on carcasses exiting the chillers, whereas in all other stages of processing the incidence was 10–20%.

In addition, Lillard (1990) reported that immersion chilling has washing effects and reduces the aerobic *Enterobacteriaceae* members. However, the incidence of *Salmonella* on post-chill carcasses was high indicating cross contamination of carcasses in chilling tanks and converting *Salmonella*-negative carcasses to positive. The same study observed a 15% and 28% increase in the incidence of *Salmonella* on post-chill carcasses compared to the pre-chill carcasses. The chilling process alone had no effect on reducing the pathogen numbers (Yang et al. 2001). The chilling process and associated water uptake also aid pathogen attachment on the skin since the process exposes deep channels and crevices on the skin (Kim et al. 1996). Along with these, aging of chilling water and increase in organic load in water reduce the efficacy of common antimicrobial agents, including chlorine and pose a significant threat for carcass contamination (Kim et al. 1996; Lillard 1990; Nagel et al. 2013; Nchez et al. 2002; Yang et al. 2001).

Currently, different antimicrobial interventions including chlorine, organic acids, essential oils, sodium hypochlorite, acetic acid, trisodium phosphate, sodium metabisulfite, and per acetic acid are applied or studied to control/eliminate NTS in poultry processing (Bucher et al. 2012; Burt 2004; Milillo and Ricke 2010; Nagel et al. 2013; Nair et al. 2014, 2015; Tamblyn et al. 1997; Tamblyn and Conner 1997; Venkitanarayanan et al. 2013). Among the different antimicrobial agents, USDA-approved safe and suitable antimicrobial agents for the application of meat, poultry, and egg products are described in the FSIS Directive (FSIS 2017). Those interventions will be dealt in detail in the subsequent chapters.

1.4 Conclusions

Intensive production of poultry in a vertically integrated system and the high consumption rate and demand for poultry meat in the USA make poultry meat a potentially important vehicle for foodborne outbreaks. Live poultry and poultry meat are commonly encountered in human salmonellosis as epidemiological links between them are understood. *Salmonella* colonization in the gastrointestinal tract of poultry and the excretion of the pathogen through droppings result in environmental contamination and contamination of poultry carcasses during processing. In a vertically integrated production system, *Salmonella* that are potentially present in the breeder flocks can be found on poultry carcasses if the intervention strategies are not effective to control the pathogen during production and processing steps. The persistence of *Salmonella* is often worsened by horizontal transmission of the pathogen by different carriers in and out of the farm and processing facilities. Therefore, vector control programs, proper biosafety measures, accurate disinfection, and intervention strategies are necessary to control *Salmonella* in poultry production systems.

References

Andino, A., Pendleton, S., Zhang, N., Chen, W., Critzer, F., & Hanning, I. (2014). Survival of *Salmonella enterica* in poultry feed is strain dependent. *Poultry Science, 93*(2), 441–447. [cited 2017 Sep 5]. Retrieved from https://academic.oup.com/ps/article-lookup/doi/10.3382/ps.2013-03401

Angulo, F. J., & Swerdlow, D. L. (1999). Epidemiology of human *Salmonella enterica* serovar enteritidis infections in the United States. In A. M. Saeed, R. K. Gast, M. E. Potter, & P. G. Wall (Eds.), *Salmonella enterica serovar enteritidis in humans and animals: Epidemiology, pathogenesis and control*. Ames, IA: Iowa State University Press. [cited 2017 Sep 6]. Retrieved from https://www.cabdirect.org/cabdirect/abstract/19992205149

Antunes, P., Mourão, J., Campos, J., & Peixe, L. (2016). Salmonellosis: The role of poultry meat. *Clinical Microbiology and Infection, 22*, 110. [cited 2017 Sep 5]. Retrieved from http://ac.els-cdn.com/S1198743X15010307/1-s2.0-S1198743X15010307-main.pdf?_tid=09c36dc4-9262-11e7-8fd5-00000aab0f01&acdnat=1504633722_6265da3f1319622904b415c62d0ce5ba

Antunes, P., Réu, C., Sousa, J. C., Peixe, L., & Pestana, N. (2003). Incidence of *Salmonella* from poultry products and their susceptibility to antimicrobial agents. *International Journal of Food Microbiology, 82*(2), 97–103. [cited 2017 Sep 5]. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/S0168160502002519

Atterbury, R. J., Van Bergen, M. A. P., Ortiz, F., Lovell, M. A., Harris, J. A., De Boer, A., et al. (2007). Bacteriophage therapy to reduce *Salmonella* colonization of broiler chickens. *Applied and Environmental Microbiology, 73*(14), 4543–4549. [cited 2017 Sep 6]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17526794

Bailey, J. S., Cox, N. A., & Berrang, M. E. (1994). Hatchery-acquired *Salmonellae* in broiler chicks. *Poultry Science, 73*(7), 1153–1157. [cited 2017 Sep 5]. Retrieved from https://academic.oup.com/ps/article-lookup/doi/10.3382/ps.0731153

Bailey, J. S., Cox, N. A., Craven, S. E., & Cosby, D. E. (2002). Serotype tracking of *Salmonella* through integrated broiler chicken operations. *Journal of Food Protection, 65*(5), 742–745. [cited 2017 Sep 5]. Retrieved from http://www.jfoodprotection.org/doi/pdf/10.4315/0362-028X-65.5.742

Barrow, P. A., & Freitas Neto, O. C. (2011). Pullorum disease and fowl typhoid—New thoughts on old diseases: A review. *Avian Pathology, 40*(1), 1–13. [cited 2017 Sep 5]. Retrieved from http://www.tandfonline.com/doi/abs/10.1080/03079457.2010.542575

Baskerville, A., Humphrey, T. J., Fitzgeorge, R. B., Cook, R. W., Chart, H., Rowe, B., et al. (1992). Airborne infection of laying hens with *Salmonella* enteritidis phage type 4. *The Veterinary Record, 130*(18), 395–398. [cited 2017 Sep 5]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1609467

Bean, N. H., & Griffin, P. M. (1990). Foodborne disease outbreaks in the United States, 1973-1987: Pathogens, vehicles, and trends. *Journal of Food Protection, 53*(9), 804–817. [cited 2017 Sep 5]. Retrieved from http://jfoodprotection.org/doi/pdf/10.4315/0362-028X-53.9.804

Berrang, M. E., Bailey, J. S., Altekruse, S. F., Shaw, W. K., Patel, B. L., Meinersmann, R. J., et al. (2009). Prevalence, serotype, and antimicrobial resistance of *Salmonella* on broiler carcasses postpick and postchill in 20 U.S. processing plants. *Journal of Food Protection, 72*(8), 1610–1615. [cited 2017 Sep 5]. Retrieved from http://www.jfoodprotection.org/doi/pdf/10.4315/0362-028X-72.8.1610

Berrang, M. E., Buhr, R. J., Cason, J. A., & Dickens, J. A. (2001). Broiler carcass contamination with *Campylobacter* from feces during defeathering. *Journal of Food Protection, 64*(12), 2063–2066. [cited 2017 Sep 5]. Retrieved from http://www.jfoodprotection.org/doi/pdf/10.4315/0362-028X-64.12.2063

Bohaychuk, V. M., Gensler, G. E., King, R. K., Manninen, K. I., Sorensen, O., Wu, J. T., et al. (2006). Occurrence of pathogens in raw and ready-to-eat meat and poultry products collected from the retail marketplace in Edmonton, Alberta, Canada. *Journal of Food Protection,*

69(9), 2176–2182. [cited 2017 Sep 5]. Retrieved from http://jfoodprotection.org/doi/pdf/10.4315/0362-028X-69.9.2176

Bucher, O., Farrar, A. M., Totton, S. C., Wilkins, W., Waddell, L. A., Wilhelm, B. J., et al. (2012). A systematic review-meta-analysis of chilling interventions and a meta-regression of various processing interventions for *Salmonella* contamination of chicken. *Preventive Veterinary Medicine, 103*(1), 1–15.

Bullis, K. L. (1977). The history of avian medicine in the U.S. II. Pullorum disease and fowl typhoid. *Avian Diseases, 21*(3), 422–429. [cited 2017 Sep 5]. Retrieved from http://www.jstor.org/stable/1589326

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods—A review. *International Journal of Food Microbiology, 94*(3), 223–253.

Bushnell, L. D., & Payne, L. F. (1932). Dissemination of pullorum disease in the incubator. In B. J. C. Te Hennepe (Ed.), *International review of poultry science. Fifth World's Poultry Congress and Exhibition, Rome* (pp. 46–48). Holland, The Netherlands: Rotterdam. Retrieved from https://naldc.nal.usda.gov/naldc/download.xhtml?id=CAT40000897&content=PDF

Callaway, T. R., Edrington, T. S., Anderson, R. C., Harvey, R. B., Genovese, K. J., Kennedy, C. N., et al. (2008). Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. *Animal Health Research Reviews, 9*(2), 217–225. [cited 2017 Sep 6]. Retrieved from https://www.cambridge.org/core/services/aop-cambridge-core/content/view/69E86594270E6E333D68F6DF88D9CF9D/S1466252308001540a.pdf/probiotics_prebiotics_and_competitive_exclusion_for_prophylaxis_against_bacterial_disease.pdf

Carrasco, E., Morales-Rueda, A., & García-Gimeno, R. M. (2012). Cross-contamination and recontamination by *Salmonella* in foods: A review. *Food Research International, 45*(2), 545–556. [cited 2017 Mar 11]. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/S0963996911006260

Cason, J. A., Cox, N. A., & Bailey, J. S. (1994). Transmission of *Salmonella* Typhimurium during hatching of broiler chicks. *Avian Diseases, 38*(3), 583. [cited 2017 Sep 5]. Retrieved from http://www.jstor.org/stable/1592082?origin=crossref

CDC. (2000). *Surveillance for foodborne disease outbreaks—United States, 1993-1997*. [cited 2017 Sep 5]. Retrieved from https://www.cdc.gov/MMWr/preview/mmwrhtml/ss4901a1.htm

CDC. (2013). *Surveillance for foodborne disease outbreaks—United States, 1998–2008*. [cited 2017 Jan 15]. Retrieved from https://www.cdc.gov/mmwr/preview/mmwrhtml/ss6202a1.htm

CDC. (2016). *Multi-state outbreak of Salmonella* Oranienburg *infections linked to shell eggs*. [cited 2017 Sep 17]. Retrieved from https://www.cdc.gov/salmonella/oranienburg-10-16/

Chalker, R. B., & Blaser, M. J. (1988). A review of human salmonellosis: Magnitude of *Salmonella* infection in the United States. *Reviews of Infectious Diseases, 10*(1), 111–124. [cited 2017 Sep 5]. Retrieved from http://www.jstor.org/stable/4454281

Chen, H.-M., Wang, Y., Su, L.-H., & Chiu, C.-H. (2013). Nontyphoid *Salmonella* infection: Microbiology, clinical features, and antimicrobial therapy. *Pediatrics and Neonatology, 54*(3), 147–152. [cited 2017 Sep 5]. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/S1875957213000119

Chittick, P., Sulka, A., Tauxe, R. V., & Fry, A. M. (2006). A summary of national reports of foodborne outbreaks of *Salmonella* Heidelberg infections in the United States: Clues for disease prevention. *Journal of Food Protection, 69*(5), 1150–1153. [cited 2017 Sep 5]. Retrieved from http://jfoodprotection.org/doi/pdf/10.4315/0362-028X-69.5.1150

Clavijo, R. I., Loui, C., Andersen, G. L., Riley, L. W., & Lu, S. (2006). Identification of genes associated with survival of *Salmonella enterica* serovar Enteritidis in chicken egg albumen. *Applied and Environmental Microbiology, 72*(2), 1055–1064. [cited 2017 Sep 5]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16461649

Clouser, C. S., Doores, S., Mast, M. G., & Knabel, S. J. (1995a). The role of defeathering in the contamination of turkey skin by *Salmonella* species and *Listeria monocytogenes*. *Poultry Science, 74*(4), 723–731. [cited 2017 Sep 6]. Retrieved from https://watermark.silverchair.com/api/watermark?token=AQECAHi208BE49Ooan9kkhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAgEwggH9B

gkqhkiG9w0BBwagggHuMIIB6gIBADCCAeMGCSqGSIb3DQEHATAeBglghkg-BZQMEAS4wEQQMPP8pXrAo5qEytwnOAgEQgIIBtAfzUrNnJTQuxEYb7yOd2_jVLuZ-Dk2rL1gqjt3HJEPxJ0

Clouser, C. S., Knabel, S. J., Mast, M. G., & Doores, S. (1995b). Effect of type of defeathering system on *Salmonella* cross-contamination during commercial processing. *Poultry Science, 74*(4), 732–741. [cited 2017 Sep 6]. Retrieved from https://academic.oup.com/ps/article-lookup/doi/10.3382/ps.0740732

Cooper, G. L., Venables, L. M., & Lever, M. S. (1996). Airborne challenge of chickens vaccinated orally with the genetically-defined *Salmonella* enteritidis aroA strain CVL30. *The Veterinary Record, 139*(18), 447–448. [cited 2017 Sep 5]. Retrieved from http://veterinaryrecord.bmj.com/cgi/doi/10.1136/vr.139.18.447

Cox, N. A., Berrang, M. E., & Cason, J. A. (2000). *Salmonella* penetration of egg shells and proliferation in broiler hatching eggs—A review. *Poultry Science, 79*(11), 1571–1574. [cited 2017 Sep 5]. Retrieved from https://academic.oup.com/ps/article-lookup/doi/10.1093/ps/79.11.1571

Crespo, R., Jeffrey, J. S., Chin, R. P., Sentíes-Cué, G., & Shivaprasad, H. L. (2004). Phenotypic and genotypic characterization of *Salmonella arizonae* from an integrated turkey operation. *Avian Diseases, 48*(2), 344–350. [cited 2017 Sep 5]. Retrieved from http://www.bioone.org/doi/abs/10.1637/7116

Cui, S., Ge, B., Zheng, J., & Meng, J. (2005). Prevalence and antimicrobial resistance of *Campylobacter* spp. and *Salmonella* serovars in organic chickens from Maryland retail stores. *Applied and Environmental Microbiology, 71*(7), 4108–4111. [cited 2017 Sep 5]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16000828

Currie, A., MaCdougall, L., Aramini, J., Gaulin, C., Ahmed, R., & Issacs, S. (2005). Frozen chicken nuggets and strips and eggs are leading risk factors for *Salmonella* Heidelberg infections in Canada. *Epidemiology and Infection, 133*(5), 809. [cited 2017 Sep 5]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16181499

Davies, R. H., & Wray, C. (1996). Persistence of *Salmonella* enteritidis in poultry units and poultry food. *British Poultry Science, 37*(3), 589–596. [cited 2017 Sep 5]. Retrieved from http://www.tandfonline.com/doi/abs/10.1080/00071669608417889

De Reu, K., Grijspeerdt, K., Messens, W., Heyndrickx, M., Uyttendaele, M., Debevere, J., et al. (2006). Eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including *Salmonella* Enteritidis. *International Journal of Food Microbiology, 112*(3), 253–260. [cited 2017 Sep 6]. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/S0168160506002303

Desmidt, M., Ducatelle, R., & Haesebrouck, F. (1997). Pathogenesis of *Salmonella* Enteritidis phage type four after experimental infection of young chickens. *Veterinary Microbiology, 56*((1-2)), 99–109. [cited 2017 Sep 5]. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/S0378113596013508

Dhanoa, A., & Fatt, Q. K. (2009). Non-typhoidal *Salmonella* bacteraemia: Epidemiology, clinical characteristics and its' association with severe immunosuppression. *Annals of Clinical Microbiology and Antimicrobials, 8*(1), 15. [cited 2017 Sep 5]. Retrieved from https://ann-clinmicrob.biomedcentral.com/track/pdf/10.1186/1476-0711-8-15?site=ann-clinmicrob.biomedcentral.com

Domınguez, C., Gómez, I., & Zumalacárregui, J. (2002). Prevalence of *Salmonella* and *Campylobacter* in retail chicken meat in Spain. *International Journal of Food Microbiology, 72*(1-2), 165–168. [cited 2017 Sep 6]. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/S0168160501006389

Donalson, L. M., Kim, W.-K., Chalova, V. I., Herrera, P., Woodward, C. L., McReynolds, J. L., et al. (2007). *In vitro* anaerobic incubation of *Salmonella enterica* serotype Typhimurium and laying hen cecal bacteria in poultry feed substrates and a fructooligosaccharide prebiotic. *Anaerobe, 13*(5-6), 208–214. [cited 2017 Sep 6]. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/S1075996407000303

Donalson, L. M., McReynolds, J. L., Kim, W. K., Chalova, V. I., Woodward, C. L., Kubena, L. F., et al. (2008). The influence of a fructooligosaccharide prebiotic combined with alfalfa molt

diets on the gastrointestinal tract fermentation, *Salmonella* Enteritidis infection, and intestinal shedding in laying hens. *Poultry Science, 87*(7), 1253–1262. [cited 2017 Sep 6]. Retrieved from https://academic.oup.com/ps/article-lookup/doi/10.3382/ps.2007-00166

Duchet-Suchaux, M., Léchopier, P., Marly, J., Bernardet, P., & Delaunay, R. (1995). Quantification of experimental *Salmonella* Enteritidis carrier state in B13 leghorn chicks. *Avian Diseases, 39*(4), 796–803. [cited 2017 Sep 5]. Retrieved from http://www.jstor.org/stable/1592416

Dutil, L., Irwin, R., Finley, R., Ng, L. K., Avery, B., Boerlin, P., et al. (2010). Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. *Emerging Infectious Diseases, 16*(1), 48–54. [cited 2017 Sep 5]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20031042

Fanelli, M. J., Sadler, W. W., & Brownell, J. R. (1970). Preliminary studies on persistence of Salmonellae in poultry litter. *Avian Diseases, 14*(1), 131. [cited 2017 Sep 5]. Retrieved from http://www.jstor.org/stable/1588564?origin=crossref

Feasey, N. A., Dougan, G., Kingsley, R. A., Heyderman, R. S., & Gordon, M. A. (2012). Invasive non-typhoidal *Salmonella* disease: An emerging and neglected tropical disease in Africa. *Lancet, 379*, 2489–2499. [cited 2017 Sep 5]. Retrieved from http://ac.els-cdn.com/S0140673611617522/1-s2.0-S0140673611617522-main.pdf?_tid=45bdafda-9260-11e7-a723-00000aacb362&acdnat=1504632964_3f1afdbdc5cb208c61499dde7731a8b5

Foley, S. L., Lynne, A. M., & Nayak, R. (2008). *Salmonella* challenges: Prevalence in swine and poultry and potential pathogenicity of such isolates. *Journal of Animal Science, 86*(14 Suppl), E149–E162. [cited 2017 Sep 5]. Retrieved from http://www.animalsciencepublications.org/publications/jas/abstracts/86/14/14

Foley, S. L., Nayak, R., Hanning, I. B., Johnson, T. J., Han, J., & Ricke, S. C. (2011). Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. *Applied and Environmental Microbiology, 77*(13), 4273–4279.

FSIS. (2014). *FSIS. Compliance guide: Modernization of poultry slaughter inspection: Amendments to chilling requirements*. [cited 2017 Sep 6]. Retrieved from https://www.fsis.usda.gov/wps/wcm/connect/7a0a728e-3b29-49e9-9c1b-ec55f2f04887/Chilling-Requirements-1014.pdf?MOD=AJPERES

FSIS. (2015). *FSIS - Compliance guideline for controlling Salmonella and Campylobacter in raw poultry*. [cited 2017 Sep 6]. Retrieved from https://www.fsis.usda.gov/wps/wcm/connect/6732c082-af40-415e-9b57-90533ea4c252/Controlling-Salmonella-Campylobacter-Poultry-2015.pdf?MOD=AJPERES

FSIS. (2017). *Table of safe and suitable ingredients: Antimicrobial update 5/25/2017*. [cited 2017 Sep 6]. Retrieved from http://www.fsis.usda.gov/wps/wcm/connect/bab10e09-aefa-483b-8be8-809a1f051d4c/7120

Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., & Van Immerseel, F. (2008). *Salmonella enterica* serovar Enteritidis genes induced during oviduct colonization and egg contamination in laying hens. *Applied and Environmental Microbiology, 74*(21), 6616–6622. [cited 2017 Sep 5]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18776023

Gast, R. K. (1994). Understanding *Salmonella* Enteritidis in laying chickens: The contributions of experimental infections. *International Journal of Food Microbiology, 21*(1-2), 107–116. [cited 2017 Sep 5]. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/0168160594902046

Gast, R. K., & Benson, S. T. (1995). The comparative virulence for chicks of *Salmonella* Enteritidis phage type 4 isolates and isolates of phage types commonly found in poultry in the United States. *Avian Diseases, 39*(3), 567–574. [cited 2017 Sep 5]. Retrieved from http://www.jstor.org/stable/1591810

Gast, R. K., & Benson, S. T. (1996). Intestinal colonization and organ invasion in chicks experimentally infected with *Salmonella* Enteritidis phage type 4 and other phage types isolated from poultry in the intestinal colonization and organ invasion in chicks experimentally infected with *Salmonella*. *Avian Diseases, 40*(4), 853–857. [cited 2017 Sep 5]. Retrieved from http://www.jstor.org/stable/1592309

Gast, R. K., Guard-Bouldin, J., & Holt, P. S. (2004). Colonization of reproductive organs and internal contamination of eggs after experimental infection of laying hens with *Salmonella*

Heidelberg and *Salmonella* Enteritidis. *Avian Diseases, 48*(4), 863–869. [cited 2017 Sep 5]. Retrieved from https://naldc.nal.usda.gov/download/36855/PDF

Gast, R. K., Guraya, R., Guard-Bouldin, J., Holt, P. S., & Moore, R. W. (2007). Colonization of specific regions of the reproductive tract and deposition at different locations inside eggs laid by hens infected with *Salmonella* Enteritidis or *Salmonella* Heidelberg. *Avian Diseases, 51*(1), 40–44. [cited 2017 Sep 5]. Retrieved from https://pubag.nal.usda.gov/pubag/downloadPDF.xhtml?id=20217&content=PDF

Gast, R. K., Mitchell, B. W., & Holt, P. S. (1998). Airborne transmission of *Salmonella* Enteritidis infection between groups of chicks in controlled-environment isolation cabinets. *Avian Diseases, 42*(2), 315. [cited 2017 Sep 5]. Retrieved from http://www.jstor.org/stable/1592482?origin=crossref

Golden, N. J., Marks, H. H., Coleman, M. E., Schroeder, C. M., Bauer, N. E., & Schlosser, W. D. (2008). Review of induced molting by feed removal and contamination of eggs with *Salmonella enterica* serovar Enteritidis. *Veterinary Microbiology, 131*(3-4), 215–228. [cited 2017 Sep 5]. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/S0378113508001053

Gregory, N. G. (2005). Recent concerns about stunning and slaughter. *Meat Science, 70*(3), 481–491. [cited 2017 Sep 5]. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/S0309174005000458

Han, J., Lynne, A. M., David, D. E., Tang, H., Xu, J., Nayak, R., et al. (2012). DNA sequence analysis of plasmids from multidrug resistant *Salmonella enterica* serotype Heidelberg isolates. *PLoS One, 7*(12), e51160. [cited 2017 Sep 5]. Retrieved from http://dx.plos.org/10.1371/journal.pone.0051160

Heyndrickx, M., Vandekerchove, D., Herman, L., Rollier, I., Grijspeerdt, K., & De Zutter, L. (2002). Routes for *Salmonella* contamination of poultry meat: Epidemiological study from hatchery to slaughterhouse. *Epidemiology and Infection, 129*, 253–265. [cited 2017 Sep 5]. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2869884/pdf/12403101.pdf

Higgins, S. E., Higgins, J. P., Wolfenden, A. D., Henderson, S. N., Torres-Rodriguez, A., Tellez, G., et al. (2008). Evaluation of a *Lactobacillus*-based probiotic culture for the reduction of *Salmonella* Enteritidis in neonatal broiler chicks. *Poultry Science, 87*(1), 27–31. [cited 2017 Sep 6]. Retrieved from https://academic.oup.com/ps/article-lookup/doi/10.3382/ps.2007-00210

Higgins, R., Malo, R., Rene-Roberge, E., & Gauthier, R. (1982). Studies on the dissemination of *Salmonella* in nine broiler-chicken flocks. *Avian Diseases, 26*(1), 26. [cited 2017 Sep 5]. Retrieved from http://www.jstor.org/stable/1590021?origin=crossref

Hindermann, D., Gopinath, G., Chase, H., Negrete, F., Althaus, D., Zurfluh, K., et al. (2017). *Salmonella enterica* serovar Infantis from food and human infections, Switzerland, 2010–2015: Poultry-related multi-drug resistant clones and an emerging ESBL producing clonal lineage. *Frontiers in Microbiology, 8*, 1322. [cited 2017 Sep 17]. Retrieved from http://journal.frontiersin.org/article/10.3389/fmicb.2017.01322/full

Hoelzer, K., Moreno Switt, A., Wiedmann, M., Werker, D., Signs, K., Davis, C., et al. (2011). Animal contact as a source of human non-typhoidal salmonellosis. *Veterinary Research, 42*(1), 34. [cited 2017 Sep 5]. Retrieved from http://www.veterinaryresearch.org/content/42/1/34

Hoffmann, M., Zhao, S., Pettengill, J., Luo, Y., Monday, S. R., Abbott, J., et al. (2014). Comparative genomic analysis and virulence differences in closely related *Salmonella enterica* serotype Heidelberg isolates from humans, retail meats, and animals. *Genome Biology and Evolution, 6*(5), 1046–1068.

Humphrey, T. J., Baskerville, A., Chart, H., Rowe, B., & Whitehead, A. (1992). Infection of laying hens with *Salmonella* Enteritidis PT4 by conjunctival challenge. *The Veterinary Record, 131*(17), 386–388. [cited 2017 Sep 5]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1455584

Hutt, F. B. (1938). The geneticist's objectives in poultry improvement. *The American Naturalist, 72*(740), 268–284. [cited 2017 Sep 5]. Retrieved from http://www.jstor.org/stable/2457554

Jackson, B. R., Griffin, P. M., Cole, D., Walsh, K. A., & Chai, S. J. (2013). Outbreak-associated *Salmonella enterica* serotypes and food commodities, United States, 1998-2008. *Emerging*

Infectious Diseases, 19(8), 1239–1244. [cited 2017 Sep 6]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23876503

Jain, S., & Chen, J. (2007). Attachment and biofilm formation by various serotypes of *Salmonella* as influenced by cellulose production and thin aggregative fimbriae biosynthesis. *Journal of Food Protection, 70*(11), 2473–2479. [cited 2017 Sep 5]. Retrieved from http://www.jfoodprotection.org/doi/pdf/10.4315/0362-028X-70.11.2473

Jørgensen, F., Bailey, R., Williams, S., Henderson, P., Wareing, D. R. A., Bolton, F. J., et al. (2002). Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw, whole chickens in relation to sampling methods. *International Journal of Food Microbiology, 76*(1-2), 151–164. [cited 2017 Sep 6]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12038572

Kim, K. Y., Frank, J. F., & Craven, S. E. (1996). Three-dimensional visualization of *Salmonella* attachment to poultry skin using confocal scanning laser microscopy. *Letters in Applied Microbiology, 22*(4), 280–282. [cited 2016 Nov 18]. Retrieved from http://doi.wiley.com/10.1111/j.1472-765X.1996.tb01161.x

Kollanoor Johny, A., Baskaran, S. A., Charles, A. S., Amalaradjou, M. A., Darre, M. J., Khan, M. I., et al. (2009). Prophylactic supplementation of caprylic acid in feed reduces *Salmonella* Enteritidis colonization in commercial broiler chicks. *Journal of Food Protection, 72*(4), 722–727. [cited 2017 Mar 26]. Retrieved from http://jfoodprotection.org/doi/pdf/10.4315/0362-028X-72.4.722

Kollanoor Johny, A., Mattson, T., Baskaran, S. A., Amalaradjou, M. A., Babapoor, S., March, B., et al. (2012). Reduction of *Salmonella enterica* serovar Enteritidis colonization in 20-day-old broiler chickens by the plant-derived compounds trans-cinnamaldehyde and eugenol. *Applied and Environmental Microbiology, 78*(8), 2981–2987. [cited 2017 Mar 11]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/22327574

Lammerding, A. M., Garcia, M. M., Mann, E. D., Robinson, Y., Ward, W. J. D., Truscott, R. B., et al. (1988). Prevalence of *Salmonella* and thermophilic *Campylobacter* in fresh pork, beef, veal and poultry in Canada. *Journal of Food Protection, 51*(1), 47–52. [cited 2017 Sep 5]. Retrieved from http://jfoodprotection.org/doi/pdf/10.4315/0362-028X-51.1.47?code=fopr-site

Lee, L. A., Puhr, N. D., Maloney, E. K., Bean, N. H., & Tauxe, R. V. (1994). Increase in antimicrobial-resistant *Salmonella* infections in the United States, 1989-1990. *The Journal of Infectious Diseases, 170*(1), 128–134. [cited 2017 Sep 6]. Retrieved from https://academic.oup.com/jid/article-lookup/doi/10.1093/infdis/170.1.128

Lestari, S. I., Han, F., Wang, F., & Ge, B. (2009). Prevalence and antimicrobial resistance of *Salmonella* serovars in conventional and organic chickens from Louisiana retail stores. *Journal of Food Protection, 72*(6), 1165–1172. [cited 2017 Sep 6]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19610326

Liebana, E., Garcia-Migura, L., Clouting, C., Clifton-Hadley, F. A., Breslin, M., & Davies, R. H. (2003). Molecular fingerprinting evidence of the contribution of wildlife vectors in the maintenance of *Salmonella* Enteritidis infection in layer farms. *Journal of Applied Microbiology, 94*(6), 1024–1029. [cited 2017 Sep 5]. Retrieved from http://doi.wiley.com/10.1046/j.1365-2672.2003.01924.x

Liljebjelke, K. A., Hofacre, C. L., Liu, T., White, D. G., Ayers, S., Young, S., et al. (2005). Vertical and horizontal transmission of *Salmonella* within integrated broiler production system. *Foodborne Pathogens and Disease, 2*(1), 90–102. [cited 2017 Sep 5]. Retrieved from http://www.liebertonline.com/doi/abs/10.1089/fpd.2005.2.90

Lillard, H. S. (1990). The impact of commercial processing procedures on the bacterial contamination and cross-contamination of broiler carcasses. *Journal of Food Protection, 53*(3), 202–207.

Logue, C. M., Sherwood, J. S., Olah, P. A., Elijah, L. M., & Dockter, M. R. (2003). The incidence of antimicrobial-resistant *Salmonella* spp. on freshly processed poultry from US Midwestern processing plants. *Journal of Applied Microbiology, 94*(1), 16–24. [cited 2017 Sep 5]. Retrieved from http://doi.wiley.com/10.1046/j.1365-2672.2003.01815.x

Maciorowski, K. G., Herrera, P., Kundinger, M. M., & Ricke, S. C. (2006). Animal feed production and contamination by foodborne *Salmonella*. *Journal für Verbraucherschutz und*

Lebensmittelsicherheit, 1(3), 197–209. [cited 2017 Sep 5]. Retrieved from http://link.springer.com/10.1007/s00003-006-0036-z

Maciorowski, K. G., Jones, F. T., Pillai, S. D., & Ricke, S. C. (2004). Incidence, sources, and control of food-borne *Salmonella* spp. in poultry feeds. *World's Poultry Science Journal, 60*(4), 446–457. [cited 2017 Sep 5]. Retrieved from https://www.cambridge.org/core/journals/world-s-poultry-science-journal/article/incidence-sources-and-control-of-food-borne-salmonella-spp-in-poultry-feeds/606CF89458709DD95ACBC1E48ECE67E0

Marder, E. P., Cieslak, P. R., Cronquist, A. B., Dunn, J., Lathrop, S., Rabatsky-Ehr, T., et al. (2017). Incidence and trends of infections with pathogens transmitted commonly through food and the effect of increasing use of culture-independent diagnostic tests on surveillance—Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 2013–2016. *MMWR. Morbidity and Mortality Weekly Report, 66*(15), 397–403. [cited 2017 Sep 6]. Retrieved from http://www.cdc.gov/mmwr/volumes/66/wr/mm6615a1.htm

McIlroy, S. G., McCracken, R. M., Neill, S. D., & O'Brien, J. J. (1989). Control, prevention and eradication of *Salmonella* Enteritidis infection in broiler and broiler breeder flocks. *The Veterinary Record, 125*(22), 545–548. [cited 2017 Sep 6]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2690452

Medeiros, M. A. N., Oliveira, D. C. N. D., Rodrigues, D. D. P., & Freitas, D. R. C. D. (2011). Prevalence and antimicrobial resistance of *Salmonella* in chicken carcasses at retail in 15 Brazilian cities. *Revista Panamericana de Salud Pública, 30*(6), 555. [cited 2017 Sep 5]. Retrieved from http://www.scielosp.org/pdf/rpsp/v30n6/a10v30n6.pdf

Meerburg, B. G., & Kijlstra, A. (2007). Role of rodents in transmission of *Salmonella* and *Campylobacter*. *Journal of the Science of Food and Agriculture, 87*(15), 2774–2781. [cited 2017 Sep 5]. Retrieved from http://doi.wiley.com/10.1002/jsfa.3004

Milillo, S. R., & Ricke, S. C. (2010). Synergistic reduction of *Salmonella* in a model raw chicken media using a combined thermal and acidified organic acid salt intervention treatment. *Journal of Food Science, 75*(2), M121–M125. [cited 2017 Sep 6].. https://doi.org/10.1111/j.1750-3841.2009.01510.x

Nagel, G. M., Bauermeister, L. J., Bratcher, C. L., Singh, M., & McKee, S. R. (2013). *Salmonella* and *Campylobacter* reduction and quality characteristics of poultry carcasses treated with various antimicrobials in a post-chill immersion tank. *International Journal of Food Microbiology, 165*(3), 281–286. https://doi.org/10.1016/j.ijfoodmicro.2013.05.016

Nair, D. V. T., Kiess, A., Nannapaneni, R., Schilling, W., & Sharma, C. S. (2015). The combined efficacy of carvacrol and modified atmosphere packaging on the survival of *Salmonella, Campylobacter jejuni* and lactic acid bacteria on Turkey breast cutlets. *Food Microbiology, 49*, 134.

Nair, D. V. T., Nannapaneni, R., Kiess, A., Schilling, W., & Sharma, C. S. (2014). Reduction of *Salmonella* on Turkey breast cutlets by plant-derived compounds. *Foodborne Pathogens and Disease, 11*(12), 981–987. [cited 2017 Mar 11]. Retrieved from http://online.liebertpub.com/doi/abs/10.1089/fpd.2014.1803

Nair, D., Thomas, J. V., & Kollanoor-Johny, A. (2016). *Propionibacterium freudenreichii* reduces cecal colonization of multidrug-resistant *Salmonella* Heidelberg in turkey poults. In *Poultry Science Association 105th Annual Meeting Abstracts* (pp. 21–22). Retrieved from http://www.poultryscience.org/psa16/abstracts/2016-PSA-Abstracts.pdf

NCC. (2017). *Per capita consumption of poultry and livestock, 1965 to estimated 2018, in pounds - The National Chicken Council*. [cited 2017 Sep 6]. Retrieved from http://www.nationalchickencouncil.org/about-the-industry/statistics/per-capita-consumption-of-poultry-and-livestock-1965-to-estimated-2012-in-pounds/

Nchez, M. X. S., Fluckey, W. M., Brashears, M. M., & McKee, S. R. (2002). Antibiotic susceptibility of *Campylobacter* spp. and *Salmonella* spp. in broilers processed in air-chilled and immersion-chilled environments. *Journal of Food Protection, 65*(6), 948–956.

Nde, C. W., McEvoy, J. M., Sherwood, J. S., & Logue, C. M. (2007). Cross contamination of Turkey carcasses by *Salmonella* species during defeathering. *Poultry Science, 86*(1), 162–167.

[cited 2017 Sep 5]. Retrieved from https://academic.oup.com/ps/article-lookup/doi/10.1093/ps/86.1.162

Newell, D. G., & Fearnley, C. (2003). Sources of *Campylobacter* colonization in broiler chickens. *Applied and Environmental Microbiology, 69*(8), 4343–4351. [cited 2017 Sep 5]. Retrieved from http://aem.asm.org/content/69/8/4343.short

Oakley, B. B., Lillehoj, H. S., Kogut, M. H., Kim, W. K., Maurer, J. J., Pedroso, A., et al. (2014). The chicken gastrointestinal microbiome. *FEMS Microbiology Letters, 360*(2), 100–112. [cited 2017 Sep 5]. Retrieved from https://academic.oup.com/femsle/article-lookup/doi/10.1111/1574-6968.12608

Park, S. H., Jarquin, R., Hanning, I., Almeida, G., & Ricke, S. C. (2011). Detection of *Salmonella* spp. survival and virulence in poultry feed by targeting the *hilA* gene. *Journal of Applied Microbiology, 111*(2), 426–432. [cited 2017 Sep 5]. https://doi.org/10.1111/j.1365-2672.2011.05054.x

Parveen, S., Taabodi, M., Schwarz, J. G., Oscar, T. P., Harter-Dennis, J., & White, D. G. (2007). Prevalence and antimicrobial resistance of *Salmonella* recovered from processed poultry. *Journal of Food Protection, 70*(11), 2466–2472. [cited 2017 Sep 6]. Retrieved from https://www.ars.usda.gov/ARSUserFiles/80720500/Poultry/33.pdf

Patterson, J., & Burkholder, K. (2003). Application of prebiotics and probiotics in poultry production. *Poultry Science, 82*(4), 627–631. [cited 2017 Sep 6]. Retrieved from https://academic.oup.com/ps/article-lookup/doi/10.1093/ps/82.4.627

Poppe, C. (2000). In C. Wray & A. Wray (Eds.), *Salmonella in domestic fowl* (p. 194). Wallingford, UK: CABI Publishing. [cited 2017 Sep 5]. Retrieved from http://dl.dairycattlecenter.com/book/SalmonellainDomesticAnimals.pdf#page=117

Poppe, C., Barnum, D. A., & Mitchell, W. R. (1986). Effect of chlorination of drinking water on experimental *Salmonella* infection in poultry. *Avian Diseases, 30*(2), 362. [cited 2017 Sep 5]. Retrieved from: http://www.jstor.org/stable/1590543?origin=crossref

Porwollik, S., Santiviago, C. A., Cheng, P., Florea, L., Jackson, S., & McClelland, M. (2005). Differences in gene content between *Salmonella enterica* serovar Enteritidis isolates and comparison to closely related serovars Gallinarum and Dublin. *Journal of Bacteriology, 187*(18), 6545–6555. [cited 2017 Sep 5]. Retrieved from: http://www.ncbi.nlm.nih.gov/pubmed/16159788

Rabsch, W., Tschäpe, H., & Bäumler, A. J. (2001). Non-typhoidal salmonellosis: Emerging problems. *Microbes and Infection, 3*(3), 237–247. [cited 2017 Sep 5]. Retrieved from http://ac.els-cdn.com/S1286457901013752/1-s2.0-S1286457901013752-main.pdf?_tid=713b4854-9272-11e7-86c1-00000aacb35e&acdnat=1504640768_4c270667355101c02cd4681f69c5da33

Renwick, S. A., Irwin, R. J., Clarke, R. C., McNab, W. B., Poppe, C., & McEwen, S. A. (1992). Epidemiological associations between characteristics of registered broiler chicken flocks in Canada and the *Salmonella* culture status of floor litter and drinking water. *The Canadian Veterinary Journal, 33*(7), 449–458. [cited 2017 Sep 5]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17424037

Rodrigue, D. C., Tauxe, R. V., & Rowe, A. B. (1990). International increase in *Salmonella* Enteritidis: A new pandemic? *Epidemiology and Infection, 105*, 21–27. [cited 2017 Sep 5]. Retrieved from https://www.cambridge.org/core/services/aop-cambridge-core/content/view/A41CC08D3D6213170721E3C22F128140/S0950268800047609a.pdf/international_increase_in_salmonella_enteritidis_a_new_pandemic.pdf

Roy, P., Dhillon, A. S., Lauerman, L. H., Schaberg, D. M., Bandli, D., & Johnson, S. (2002). Results of *Salmonella* isolation from poultry products, poultry, poultry environment, and other characteristics. *Avian Diseases, 46*(1), 17–24. [cited 2017 Sep 5]. Retrieved from http://www.bioone.org/doi/abs/10.1637/0005-2086%282002%29046%5B0017%3AROSIFP%5D2.0.CO%3B2

Russell, S. M. (2008). Chemical residuals in the environment and on chicken carcasses associated with scalding chickens in an acidic, copper sulfate-based commercial sanitizer during poultry processing. *Journal of Food Protection, 71*(1), 226–230.

Scharff, R. L. (2012). Economic burden from health losses due to foodborne illness in the United States. *ProQuest*. [cited 2017 Sep 5]. Retrieved from https://search.proquest.com/docview/913142449?pq-origsite=gscholar

Schleifer, J. H., Juven, B. J., Beard, C. W., & Cox, N. A. (1984). The susceptibility of chicks to *Salmonella* Montevideo in artificially contaminated poultry feed. *Avian Diseases, 28*(2), 497. [cited 2017 Sep 5]. Retrieved from http://www.jstor.org/stable/1590357?origin=crossref

Shah, D. H., Zhou, X., Addwebi, T., Davis, M. A., Orfe, L., Call, D. R., et al. (2011). Cell invasion of poultry-associated *Salmonella enterica* serovar Enteritidis isolates is associated with pathogenicity, motility and proteins secreted by the type III secretion system. *Microbiology, 157*(5), 1428–1445. [cited 2017 Sep 5]. Retrieved from http://www.microbiologyresearch.org/docserver/fulltext/micro/157/5/1428_mic044461.pdf?expires=1504644873&id=id&accname=guest&checksum=4EA85DB3BE33EA6144E037CB5C48641C

Shivaprasad, H. L. (2000). Fowl typhoid and pullorum disease. *Revue Scientifique et Technique, 19*(2), 405–424. [cited 2017 Sep 5]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10935271

Skov, M. N., Madsen, J. J., Rahbek, C., Lodal, J., Jespersen, J. B., Jørgensen, J. C., et al. (2008). Transmission of *Salmonella* between wildlife and meat-production animals in Denmark. *Journal of Applied Microbiology, 105*(5), 1558–1568. [cited 2017 Sep 5]. https://doi.org/10.1111/j.1365-2672.2008.03914.x

Slader, J., Domingue, G., Jørgensen, F., McAlpine, K., Owen, R. J., Bolton, F. J., et al. (2002). Impact of transport crate reuse and of catching and processing on *Campylobacter* and *Salmonella* contamination of broiler chickens. *Applied and Environmental Microbiology, 68*(2), 713–719. [cited 2017 Sep 5]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11823211

Stafseth, H. J., & Mallmann, W. L. (1928). Progress report on poultry disease investigation at the Michigan experiment station. *Poultry Science, 8*(1), 19–22. [cited 2017 Sep 5]. Retrieved from https://academic.oup.com/ps/article-lookup/doi/10.3382/ps.0080019

Svobodová, I., Bořilová, G., Hulánková, R., & Steinhauserová, I. (2012). Microbiological quality of broiler carcasses during slaughter processing. *Acta Veterinaria, 81*(1), 37–42. [cited 2017 Sep 6]. Retrieved from http://actavet.vfu.cz/81/1/0037/

Tamblyn, K. C., & Conner, D. E. (1997). Bactericidal activity of organic acids against *Salmonella* Typhimurium attached to broiler chicken skin. *Journal of Food Protection, 60*(6), 629–633.

Tamblyn, K. C., Conner, D. E., & Bilgili, S. F. (1997). Utilization of the skin attachment model to determine the antibacterial efficacy of potential carcass treatments. *Poultry Science, 76*(9), 1318–1323. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9276898

Tauxe, R. V., Cavanagh, T. R., & Cohen, M. L. (1989). Interspecies gene transfer *in vivo* producing an outbreak of multiply resistant shigellosis. *The Journal of Infectious Diseases, 160*, 1067–1070. [cited 2017 Sep 5]. Retrieved from https://watermark.silverchair.com/api/watermark?token=AQECAHi208BE49Ooan9kkhW_Ercy7Dm3ZL_9Cf3qfKAc485ysgAAAg0wggIJBgkqhkiG9w0BBwaggH6MIIB9gIBADCCAe8GCSqGSIb3DQEHATAeBglghkgBZQMEAS4wEQQMJHjVYc9MYCgAb14_AgEQgIIBwNPvoDvithlNDqVUjz3greFphY1_8SV692TTzjCWIIYG

Tellez, G., Pixley, C., Wolfenden, R. E., Layton, S. L., & Hargis, B. M. (2012). Probiotics/direct fed microbials for *Salmonella* control in poultry. *Food Research International, 45*(2), 628–633. [cited 2017 Sep 6]. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/S0963996911002067

Tirado, C., & Schmidt, K. (2001). WHO surveillance programme for control of foodborne infections and intoxications: Preliminary results and trends across Greater Europe. *The Journal of Infection, 43*, 80. [cited 2017 Sep 5]. Retrieved from http://ac.els-cdn.com/S0163445301908618/1-s2.0-S0163445301908618-main.pdf?_tid=047937c4-925b-11e7-8e39-00000aab0f27&acdnat=1504630707_e9e8acdcf8bd25a720ce46c4b2556730

Tittsler, R. P. (1930). Septicemic *Salmonella* Pullorum infection in adult fowls. *Poultry Science, 10*(1), 17–23. [cited 2017 Sep 5]. Retrieved from https://academic.oup.com/ps/article-lookup/doi/10.3382/ps.0100017

Tizard, I. (2004). Salmonellosis in wild birds. *Seminars in Avian and Exotic Pet Medicine, 13*(2), 50–66. [cited 2017 Sep 5]. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/S1055937X04000039

Van Immerseel, F., De Buck, J., Boyen, F., Bohez, L., Pasmans, F., Volf, J., et al. (2004b). Medium-chain fatty acids decrease colonization and invasion through *hilA* suppression shortly after infection of chickens with *Salmonella enterica* serovar Enteritidis. *Applied and Environmental Microbiology, 70*(6), 3582–3587. [cited 2017 Sep 5]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15184160

Van Immerseel, F., De Buck, J., Pasmans, F., Bohez, L., Boyen, F., Haesebrouck, F., et al. (2004a). Intermittent long-term shedding and induction of carrier birds after infection of chickens early posthatch with a low or high dose of *Salmonella* Enteritidis. *Poultry Science, 83*(11), 1911–1916. [cited 2017 Sep 5]. Retrieved from https://academic.oup.com/ps/article-lookup/doi/10.1093/ps/83.11.1911

Van Immerseel, F., Russell, J. B., Flythe, M. D., Gantois, I., Timbermont, L., Pasmans, F., et al. (2006). The use of organic acids to combat *Salmonella* in poultry: A mechanistic explanation of the efficacy. *Avian Pathology, 35*(3), 182–188. [cited 2016 Oct 15]. Retrieved from http://www.tandfonline.com/doi/full/10.1080/03079450600711045

Velge, P., Cloeckaert, A., & Barrow, P. (2005). Emergence of *Salmonella* epidemics: The problems related to *Salmonella enterica* serotype Enteritidis and multiple antibiotic resistance in other major serotypes. *Veterinary Research, 36*, 267–288. [cited 2017 Sep 5]. Retrieved from https://www.vetres.org/articles/vetres/pdf/2005/03/v4058.pdf

Venkitanarayanan, K., Kollanoor-Johny, A., Darre, M. J., Donoghue, A. M., & Donoghue, D. J. (2013). Use of plant-derived antimicrobials for improving the safety of poultry products. *Poultry Science, 92*(2), 493–501.

Waldroup, A. L. (1996). Contamination of raw poultry with pathogens. *World's Poultry Science Journal, 52*(1), 20–25. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-22944471495&partnerID=40&md5=aac074e5fa41a0e011d6ae897f349d62

Wales, A., Breslin, M., Carter, B., Sayers, R., & Davies, R. (2007). A longitudinal study of environmental *Salmonella* contamination in caged and free-range layer flocks. *Avian Pathology, 36*(3), 187–197. [cited 2017 Sep 5]. Retrieved from http://www.tandfonline.com/doi/full/10.1080/03079450701338755

White, D. G., Zhao, S., Sudler, R., Ayers, S., Friedman, S., Chen, S., et al. (2001). The isolation of antibiotic-resistant *Salmonella* from retail ground meats. *The New England Journal of Medicine, 345*(16), 1147–1154. [cited 2017 Sep 5]. Retrieved from http://www.nejm.org/doi/abs/10.1056/NEJMoa010315

Williams, J. E., & Benson, S. T. (1978). Survival of *Salmonella* Typhimurium in poultry feed and litter at three temperatures. *Avian Diseases, 22*(4), 742. [cited 2017 Sep 5]. Retrieved from http://www.jstor.org/stable/1589652?origin=crossref

Winter, S. E., Thiennimitr, P., Winter, M. G., Butler, B. P., Huseby, D. L., Crawford, R. W., et al. (2010). Gut inflammation provides a respiratory electron acceptor for *Salmonella*. *Nature, 467*(7314), 426–429. [cited 2017 Sep 5]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20864996

Wong, M. H. Y., Yan, M., Chan, E. W. C., Biao, K., & Chen, S. (2014). Emergence of clinical *Salmonella enterica* serovar Typhimurium isolates with concurrent resistance to ciprofloxacin, ceftriaxone, and azithromycin. *Antimicrobial Agents and Chemotherapy, 58*(7), 3752–3756. [cited 2017 Sep 17]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24752251

Yang, H., Li, Y. B., & Johnson, M. G. (2001). Survival and death of *Salmonella* Typhimurium and *Campylobacter jejuni* in processing water and on chicken skin during poultry scalding and chilling. *Journal of Food Protection, 64*(6), 770–776.

Yim, L., Betancor, L., Martinez, A., Giossa, G., Bryant, C., Maskell, D., et al. (2010). Differential phenotypic diversity among epidemic-spanning *Salmonella enterica* serovar Enteritidis isolates from humans or animals. *Applied and Environmental Microbiology, 76*(20), 6812–6820. [cited 2017 Sep 5]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/20802078

Zhang-Barber, L., Turner, A. K., & Barrow, P. A. (1999). Vaccination for control of *Salmonella* in poultry. *Vaccine, 17*(20-21), 2538–2545. [cited 2017 Sep 6]. Retrieved from http://linkinghub.elsevier.com/retrieve/pii/S0264410X99000602

Zhao, S., White, D. G., Friedman, S. L., Glenn, A., Blickenstaff, K., Ayers, S. L., et al. (2008). Antimicrobial resistance in *Salmonella enterica* serovar Heidelberg isolates from retail meats, including poultry, from 2002 to 2006. *Applied and Environmental Microbiology, 74*(21), 6656–6662. [cited 2017 Sep 6]. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18757574

Chapter 2
Reducing Foodborne Pathogens in Organic Poultry: Challenges and Opportunities

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2.1 Introduction

Organic poultry production is becoming increasingly popular in the United States with approximately 17% increase in the sales of organic meat and poultry, contributing $991 million in 2016 (OTA 2017). Even though the production costs for organic meat are higher compared to conventional poultry, the net income from sales of organic chicken meat is also significantly higher than the conventional production (Cobanoglu et al. 2014), which makes it profitable for the producers. Since the central philosophy of organic agriculture is to reduce the impact of agriculture practices on animals, humans, and the environment (IFOAM 2009), organic farming restricts the use of synthetic compounds (e.g., antibiotics, hormones, pesticides, and herbicides) in agricultural production (Berg 2002; Harper and Makatouni 2002; Lund and Algers 2003; Fanatico 2006; Kijlstra and Eijck 2006; Lund 2006; Jacob et al. 2008; Fanatico et al. 2009). According to the Organic Farming Research Foundation (OFRF), complying with the organic standards is one of the most pressing needs of organic livestock and poultry production (OFRF 2007).

Salmonella and *Campylobacter* are two major foodborne pathogens epidemiologically linked to the consumption of chicken and eggs which together account for most of the laboratory-confirmed cases of bacterial gastroenteritis in the United States (Scallan et al. 2011; CDC 2017a). Although the conventional poultry industry

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is equipped with several interventions to control these pathogens on meat and eggs, organic poultry producers have access to only a limited number of antibacterials (e.g., weak organic acids, chlorine, oxidizing compounds) that are safe, effective, and approved for improving the product safety and shelf life of poultry meat and/or eggs (Taylor et al. 2012). Although organic food products may represent a safer alternative with regard to chemical contamination of the product, control of foodborne pathogens in organic poultry is particularly important because consumers of these products perceive them as being safer and choose them for children, the elderly, and immunocompromised people (Magkos et al. 2003). This is a concern for organic producers because they cannot control proper cooking and other food safety practices of consumers once the poultry products are sold.

This chapter discusses the food safety challenges and potential strategies to reduce pathogens both in preharvest and postharvest conditions while conforming to organically approved methods. Challenges unique to organic production such as required outdoor access and availability of certified feed ingredients are also discussed.

2.2 Challenges for Organic Poultry Producers

In spite of the growing popularity for the organic products, there is limited scientific literature related to health and welfare of poultry raised on organic production systems. There are several speculations about superior flavor, health benefits, and safety of organic food products; however, there is little scientific evidence to confirm or dismiss such claims (Kijlstra and Eijck 2006). The central philosophy of organic agriculture is to reduce the impact of agriculture practices on animals, humans, and the environment (IFOAM 2009). Based on the available literature, the key challenges for organic poultry producers are associated with:

1. Animal health and food safety implications
2. Access to outdoors
3. Slaughter and processing issues

2.2.1 Animal Health and Food Safety Implications

Foodborne illness is a crisis, and each year an estimated 48 million Americans (1 in 6) become ill from consuming contaminated foods or beverages (CDC 2015). *Campylobacter* and *Salmonella* spp. are two major foodborne pathogens that account for the majority of all reported cases of bacterial foodborne illness in 2016 (CDC 2017a). Both *Campylobacter* and *Salmonella* infections in humans are often associated with improper handling/consumption of contaminated chicken and/or eggs (Painter et al. 2013). The incidence of *Salmonella* and *Campylobacter* has

been reported from both conventional and organic production systems (Andrews and Baumler 2005; Nachamkin and Guerry 2005; Novak et al. 2005; Tuyttens et al. 2008; Young et al. 2009). Although the conventional poultry industry is equipped with several interventions to control these pathogens on meat and eggs, organic poultry producers have access to only a limited number of antibacterials that are safe, effective, and approved for improving the product safety and shelf life of poultry meat and/or eggs. Recent outbreaks and recalls due to contamination with foodborne pathogens in organic poultry products demonstrate the need to control these pathogens in organic poultry (Noyes 2009; FDA 2011; Anonymous 2011, 2014, 2016). Chickens can become exposed to pathogens in many ways, among them by eating insects that pick up bacteria from the environment (e.g., dark and dung beetles are a known vector for *Campylobacter*), pecking at droppings that carry germs (i.e., *Clostridium*, *Salmonella*, and *Campylobacter* can readily colonize the gastrointestinal tract of multiple animal species), as well as exposure to contaminated soil and water, where these pathogens can survive for several weeks or months depending on the weather and other environmental conditions. However, chickens are the reservoir host of *S. enteritidis* and *C. jejuni*, with their intestinal colonization being the single most significant factor causing contamination of meat and eggs. The primary colonization site of *Salmonella* in chickens is the ceca (Andreatti Filho et al. 2000; Stern 2008), with cecal carriage of *Salmonella* leading to horizontal transmission of the infection, contamination of carcasses and eggshell with feces, and potential retrocontamination of the ovaries (Gantois et al. 2009). Egg contamination with *S. enteritidis* results by penetration through the eggshell from contaminated feces during or after oviposition (Gast and Beard 1990; Messens et al. 2005, 2006). Similarly, *C. jejuni* primarily colonizes the mucus overlying the epithelial cells in the ceca and small intestine of chickens. In broiler chickens, *C. jejuni* colonization can persist during the entire life span of birds, thus resulting in carcass contamination during slaughter (Lin 2009).

Organic poultry products have been associated with multiple outbreaks due to *S. enteritidis*. In 2009, organic brown eggs contaminated with *Salmonella* were recalled in California (Noyes 2009). In 2011, a multistate *S. enteritidis* outbreak was attributed to contaminated organic eggs causing illnesses in children and adults (FDA 2011). State public health authorities traced the outbreak to a single organic farm. In 2014, certified organic eggs were recalled owing to a potential contamination due to *Salmonella* (Anonymous 2011). In addition, a multistate recall of organic eggs tainted with *Salmonella* was reported in 2014 (Anonymous 2014). The latest outbreak was in January 2016, wherein the FDA reported a voluntary recall of shelled eggs, including those from free-range birds, due to contamination with *Salmonella* (Anonymous 2016; FDA 2016). Although the number of people sickened and/or hospitalized is yet to be confirmed, severe loss of production at both the retail and wholesale level was reported. According to recent CDC report (2017b), multistate outbreaks of human *Salmonella* infections were linked to contact with backyard poultry, resulting in 961 cases, 215 hospitalizations, and 1 death from January 2017 to July 2017. These outbreaks and recalls underscore the need for effective methods to improve postharvest food safety of organic poultry products.

2.2.2 Access to Outdoors

In organic poultry production, there are multiple avenues where the birds become colonized with pathogens. This is because the birds have access to the outdoors, where they can encounter pathogens from wild animals and, at times, stress from environmental conditions such as temperature extremes and predation (Sundrum 2001; Engvall 2002). Bacterial pathogens such as *Salmonella* and *Campylobacter* are frequently present in the soil and water and can infect birds and subsequently humans through foodborne transmission. Also, multispecies grazing is one of the key features of an ecologically centered organic production, and poultry are often raised with other farm animals. However, this integration additionally exposes birds to contact with manure of other animals, where many pathogens survive, and increase chances for colonization with these pathogens. Bailey and Cosby (2005) studied *Salmonella* in free-range and certified organic chicken and found that 64% of 14 lots and 31% of 135 carcasses were positive for this pathogen. Studies comparing the prevalence of foodborne pathogens found no significant differences in the prevalence of *Salmonella* between organic and conventionally raised broilers; however, *Campylobacter* colonization in organic flocks was higher compared to conventional broiler flocks in a few studies (Cui et al. 2005; Van Overbeke et al. 2006).

2.2.3 Slaughter and Processing Issues

Several strategies have been developed to reduce enteric pathogens in preharvest poultry with varying degrees of success. However, these strategies do not ensure complete inhibition of pathogens. This reiterates the need for developing scientifically validated and novel strategies for controlling pathogens in postharvest organic poultry production. One of the challenges with the organic poultry processing involves organically approved procedures for handling, slaughter, and processing of poultry (Federal Register 2017). Unfortunately, organic poultry processors have very limited strategies that are safe, effective, and approved for reducing pathogens on poultry carcass and/or eggs, other than chlorine and peracetic acid. However, recent recommendations from the National Organic Coalition (NOC) suggest minimizing the use of chlorine and develop viable alternatives to ensure safety of organically processed poultry and poultry products. Moreover, immersion and spraying of poultry carcasses with chlorine are not very effective in reducing *Salmonella* due to the presence of large amounts of organic matter such as blood and feces (Hargis et al. 1998; FSIS 2015). In addition, formation of harmful chemical by-products such as chloramines, trihalomethanes, and other organochlorine compounds when chlorine and organic materials interact is a concern due to potential health risks, including cancer (Richardson et al. 1998; Donato and Zani 2010; Dore 2015). Therefore, alternate, safe, and effective postharvest antibacterial interventions are needed by the organic poultry industry for reducing pathogens on chicken carcasses and eggs.

Although pathogens in foods are destroyed by proper cooking, it is important to reduce pathogenic microbes in raw food products such as poultry for preventing cross-contamination and microbial proliferation in the kitchen and food service areas (Ravishankar et al. 2008; Brown et al. 2013; Oscar 2013). For cooked products, some consumers may inadvertently not fully cook these foods, thereby posing a food safety concern. For example, a large 2011 *Salmonella* outbreak linked to organic eggs was due to improper cooking of the eggs (http://www.outbreakdatabase.com/details/larry-schultz-organic-farms-eggs-2011/?year=2011). As noted earlier, organic foods are perceived by consumers as safer than conventional foods, thereby increasing the responsibility on organic producers and processors to ensure product safety and meet consumer expectations.

2.3 Reducing the Prevalence of Pathogens in Organic Chickens

To control foodborne pathogens and other diseases, organic poultry producers rely primarily on biosecurity and management strategies (such as keeping the animals in portable pens that can be frequently moved to a clean spot) and a system approach program that promotes adequate immune system development and gut health (Fanatico 2006; OACC 2008). However, when problems develop within organic facilities, producers have few well-researched options for prevention and therapeutic treatment (OACC 2008). This situation has forced many organic producers to use practices that appear to be anecdotal rather than scientifically proven treatments (OFRF 2007). For example, one of the most well-known sources of information about remedies for organic poultry is the one produced by Karma Glos (2011). In this compendium, the author recommends remedies such as copper sulfate and formic acid to treat enteric problems, but these compounds are known to have adverse effects on the health of the animals (such as causing lesions in the mouth and crop and reducing feed absorption; Chaveerach et al. 2002). The author, as well as numerous poultry producers, often uses natural preparations such as garlic water or adding turmeric to the feed (Padgham 2006). However, these remedies are better known for their anti-inflammatory properties than their antibacterial effectiveness and have not been systematically evaluated for effectiveness against poultry pathogens. In addition, recommendations such as moving the animals to fresh pastured areas frequently are not practical solutions as many producers have permanent buildings or confine their animals at night, and since most animals will defecate during the night or early in the morning, exposure to pathogens can be very high in these buildings.

There has been an increasing interest in exploring the efficacy of natural compounds for controlling foodborne pathogens in poultry. Some of the extensively studied natural strategies with efficacy against both animal and human pathogens are natural plant extracts, organic acids, prebiotics, and probiotics (Chaveerach et al. 2002; Friedman et al. 2004; Gill and Holley 2004; van Immerseel et al. 2004a, b; Prabuseenivasan et al. 2006; Thormar et al. 2006; Cox and Markham 2007; Shahverdi et al. 2007). Some of the major advantages of using the aforementioned

strategies include quick biodegradability, environmentally sustainable, and they are natural. Therefore, there is higher likelihood of these approaches being accepted by both the organic producers and consumers, and at the same time, they are suitable for inhibiting pathogens in organic production systems.

2.3.1 *Plant Extracts as Safe and Effective Treatments for Organic Poultry*

With the increasing consumer preference toward natural products, using plant based products as potential alternatives to antibiotics has increased (Gauthier 2003). Plant extracts and essential oils are important components of most traditional medical systems (Wollenweber 1988), generally regarded as safe by the FDA (21 CFR 182.20, FDA 2017), and are approved for use in food animals. Historically, plants have served as a source for the development of novel drugs, thereby contributing to human health and well-being. Plants are capable of synthesizing a large number of molecules, most of which are phenolic compounds or their derivatives (Geissman 1963). A majority of these compounds are naturally produced by plants as a defense mechanism against predation by microorganisms and insects. More than 5000 plant phenolics and polyphenols have been identified, many of which possess a wide spectrum of biological effects, including anti-inflammatory, antimicrobial, anticarcinogenic, cardioprotective, and neuroprotective properties (Beretz et al. 1978; Wollenweber 1988). Generally, the natural plant extracts with the strongest antimicrobial properties contain a high percentage of phenolic compounds such as eugenol, thymol, and aldehyde compounds, such as trans-cinnamaldehyde (Burt 2004). All of these compounds have shown in vitro and in vivo effectiveness against bacteria such as *E. coli*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Salmonella typhimurium*, and different *Clostridium* spp. (Dorman and Deans 2000; Mitsch et al. 2004; Si et al. 2006). Plant extracts include trans-cinnamaldehyde, an aldehyde present in the bark of cinnamon (*Cinnamomum zeylandicum*), and thymol and carvacrol, phenolic compounds obtained from plant sources such as origanum oil (*Origanum vulgare*) and oil of thyme (*Thymus vulgaris*). Eugenol is another compound that has been extensively studied and is an active ingredient extracted from cloves. β-Resorcylic acid (2,4 dihydroxybenzoic acid) is yet another phytophenolic compound that is widely distributed among many plants as a secondary metabolite to protect plants against pathogens (Friedman et al. 2003). These compounds are accepted as "Generally recognized as safe" (GRAS) by the FDA (21 CFR 182.60) and have demonstrated beneficial effects to gut health and significant antimicrobial properties. These compounds are relatively inexpensive; even though the costs are not fixed, according to a recent report by Darre et al. (2014), the cost for caprylic acid is ~$7.68/kg, clove oil ~$3.78/kg, carvacrol ~$48.40/kg, cinnamaldehyde ~$4.40/kg, and thymol $484/kg. Additionally, due to their GRAS status, they are approved for use in organic agriculture in accordance to the National Organic Program through the National List of Allowed and Prohibited Substances (NOP 2009).

A brief description of these compounds is presented below.

Trans-cinnamaldehyde is an aldehyde, naturally found as the principal ingredient in cinnamon oil (*Cinnamomum verum)*. Trans-cinnamaldehyde is commonly used in agriculture to prevent fungal infections in crops (PAN 2007) and also has been reported to possess antimicrobial activity toward a wide range of pathogens, including *Aspergillus* spp. (Yin et al. 2015), *Clostridium botulinum* (Bowles and Miller 1993), *Clostridium perfringens* (Si et al. 2009), *Staphylococcus aureus* (Shen et al. 2015), *E. coli* O157:H7 (Baskaran et al. 2016), *Campylobacter jejuni* (Johny et al. 2008), *Listeria monocytogenes* (Upadhyay et al. 2012), and *Salmonella enterica* (Upadhyaya et al. 2015). It has been proposed that trans-cinnamaldehyde interferes with protein binding, causes membrane disruption, and increases cell permeability leading to leakage of cellular contents (Burt 2004; Yossa et al. 2014). Research from our laboratories has demonstrated that in-feed supplementation of trans-cinnamaldehyde is effective in reducing *Salmonella enteritidis* colonization in commercial, market-age broiler chickens (Kollanoor Johny et al. 2012). Also, trans-cinnamaldehyde (TC) as in-feed supplements reduces the carriage of *Salmonella* Heidelberg (SH) in broiler chickens (Upadhyaya et al. 2016).

Carvacrol is a major constituent of oregano oil and has been reported to possess antibacterial, antiparasitic, antifungal, anti-inflammatory, antioxidative, cardioprotective, neuroprotective, and anticarcinogenic properties (Friedman 2014). Carvacrol has demonstrated efficacy against major foodborne pathogens, in vitro, and in foods including salads, fruit juices, seafood, meat, and meat products (Friedman 2014). It has been shown that carvacrol acts by altering the membrane potential, increasing the membrane permeability to protons and ions, and eventually leading to death of target organisms (Xu et al. 2008; Friedman 2014; Arsi et al. 2014).

Thymol, another GRAS compound (21CFR172.515) which is structurally similar to carvacrol, is obtained from plants belonging to Lamiaceae family such as *Origanum glandulosum*, *Thymus vulgaris*, and *Satureja* spp. (Marchese et al. 2016). Thymol is known to possess antibacterial, antifungal, and antioxidant properties and has been extensively used in food industry as a flavoring and preservative agent (Marchese et al. 2016). Research studies have demonstrated the antimicrobial activity of thymol against gram-positive and gram-negative bacteria including selected antibiotic-resistant bacteria (Palaniappan and Holley 2010; Marchese et al. 2016). The in vitro and in vivo efficacy of thymol against *Campylobacter*, *Salmonella*, and *Listeria* has been investigated extensively (Kollanoor Johny et al. 2010; Arsi et al. 2014; Upadhyay et al. 2013). Results from multiple studies performed over the last decade suggest that the potential benefit of thymol and thymol-rich essential oils is to reduce foodborne pathogens in organic poultry and poultry products (Arsi et al. 2017).

Eugenol is a major component of the oil from cloves (*Syzygium aromaticum*), commonly used as an analgesic and antiseptic, and has antibacterial properties against many microorganisms, including *Listeria monocytogenes*, *Salmonella typhimurium*, *Campylobacter*, *E. coli* O157:H7, and *Clostridium perfringens* (Blaszyk and Holley 1998; Burt 2004; Si et al. 2006, 2009; Ayoola et al. 2008). Similar to other essential oils, eugenol being hydrophobic may act by integrating into cellular and mitochondrial membranes, disrupting their function and increasing their

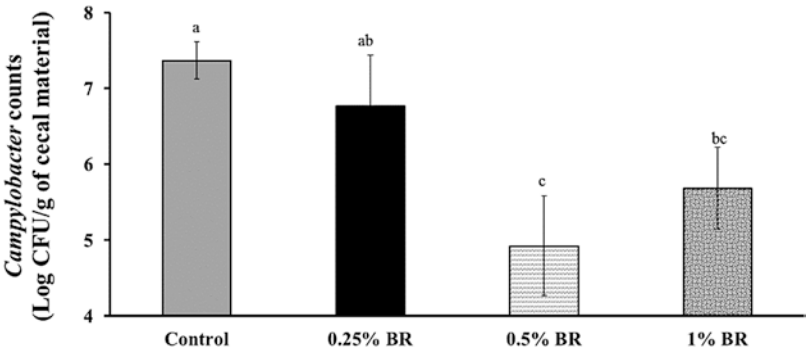

Fig. 2.1 Effect of β-resorcylic acid (BR) on cecal *Campylobacter jejuni* counts in 14-day-old broiler chickens. Results are averages of two independent experiments, each containing ten birds/treatments (mean and SEM). Bars with different letters represent a significant difference between treatments ($P < 0.05$). Reprinted with permission from Wagle et al. (2017a)

permeability (Prabuseenivasan et al. 2006). Research has demonstrated that eugenol supplemented through feed could reduce *Salmonella enteritidis* colonization in market-age chickens (Kollanoor Johny et al. 2012) and on eggs (Upadhyaya et al. 2013).

β-Resorcylic acid (BR) is a phytophenolic compound, present as a secondary metabolite, and is widely distributed among many angiosperms to protect plants against pathogens (Friedman et al. 2003). It is classified under "Everything Added to Food in the United States" by the US Food and Drug Administration (FDA 2013). β-Resorcylic acid is effective in reducing major foodborne pathogens, including *Salmonella* (Mattson et al. 2011; Upadhyaya et al. 2016), *Listeria monocytogenes* (Upadhyay et al. 2013), *Escherichia coli* O157:H7 (Baskaran et al. 2013), and *Campylobacter jejuni* (Fig. 2.1, Wagle et al. 2017a, b) in food products.

Even though plant-derived compounds showed promising results in inhibiting foodborne pathogens, further studies are needed to determine maximum effective doses and the most effective dose delivery systems (e.g., feed, water) to reduce foodborne pathogens in organically raised broiler chickens.

2.3.2 Postharvest Utilization of Plant-Derived Compounds

Currently there are no consistently effective treatments to reduce or eliminate all pathogens from colonizing poultry during rearing (Lin 2009). Although many of the plant extracts discussed above are effective for *Salmonella*, *Campylobacter* continues to be a challenge. Predictive modelling has determined that if *Campylobacter* counts can be reduced by 2 $\log_{10}$ on the postharvest poultry carcass, the incidence of human campylobacteriosis would be reduced 30-fold (Rosenquist et al. 2003). Procedures to decontaminate carcasses during processing need to be economically feasible, safe, and easily implemented into the production scheme, not detrimental to

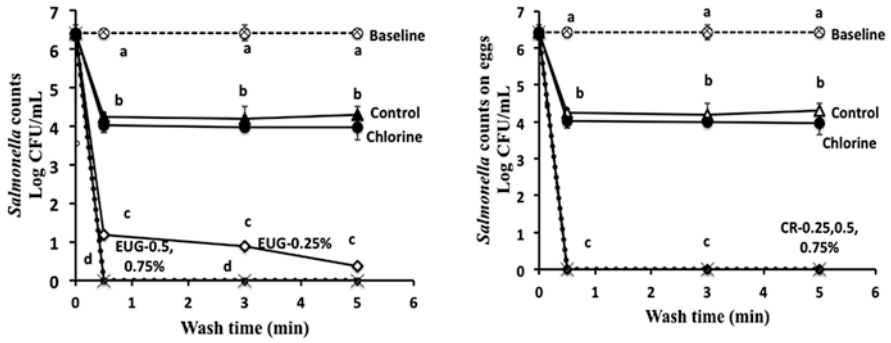

Fig. 2.2 Efficacy of eugenol (EUG) and carvacrol (CR) as a wash for reducing *Salmonella* on eggs. Reprinted with permission from Upadhyaya et al. (2013)

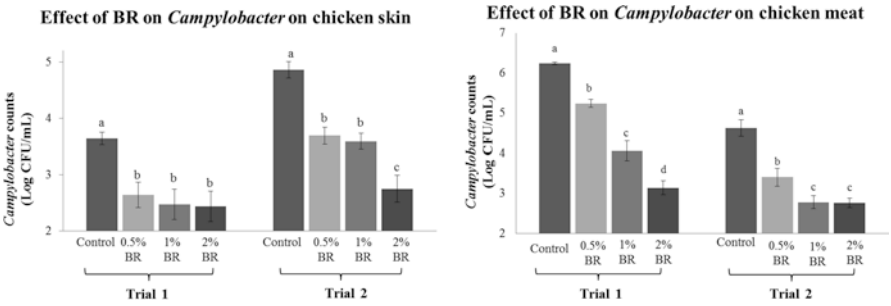

Fig. 2.3 Effect of β-resorcylic acid (BR) on *Campylobacter jejuni* counts on chicken skin and meat samples. Reprinted with permission from Wagle et al. (2017b)

treatment of waste water and not result in negative attributes to the final meat product (Loretz et al. 2010). Previous research from our laboratories have evaluated antimicrobial washes with plant compounds on meat and eggs in an attempt to reduce contamination and provide organic producer options other than chemical decontamination, for example, rapidly inactivating *S. enteritidis* on eggs to below detection limit utilizing a eugenol or carvacrol wash (Fig. 2.2, Upadhyaya et al. 2013). Similar reduction was noticed when β-resorcylic acid was used as a wash for reducing *Campylobacter jejuni* counts on chicken skin and meat (Fig. 2.3, Wagle et al. 2017b).

2.3.3 Organic Acids

Organic acids, mainly medium-chain fatty acids, have been reported to possess antibacterial activity against a wide range of pathogens (Petschow et al. 1996; Van Immerseel et al. 2004a; Nair et al. 2005; Vasudevan et al. 2005; Thormar et al. 2006; Skřivanová et al. 2006; Solis de los Santos et al. 2008, 2009; Johny et al. 2009).

Caprylic acid, also referred as octanoic acid, is an eight-carbon-long, medium-chain fatty acid which occurs naturally in milk and coconut oil (Sprong et al. 2001; Jensen 2002). It is relatively inexpensive and available in its natural form, thus making its use practical for organic farmers (Darre et al. 2014). In addition, previous studies on the use of this compound in poultry have revealed that caprylic acid exerts a significant antimicrobial effect in chickens with no toxicity to the birds. (Solis de los Santos et al. 2008, 2009, 2010). Likewise, caprylic acid in feed was effective in reducing the colonization *Salmonella enteritidis* in chicken ceca, small intestine, crop, cloaca, liver, and spleen (Johny et al. 2009). It is also important to note that caprylic acid supplementation did not significantly affect the feed intake and weight gain in birds (Johny et al. 2009). Similar to these results, van Immerseel et al. (2004a) found that feeding of medium-chain fatty acids, including caprylic acid, to chicks decreased the colonization of *S. enteritidis* in the birds. Caprylic acid was also parasiticidal against *Cryptocaryon irritans*, *Benedenia seriolae*, and *Kudoa shiomitsui* in vitro (Hirazawa et al. 2001a). In another study, while investigating the antihelminthic effect of medium-chain fatty acids against monogenean *Heterobothrium okamotoi* in the tiger puffer, investigators found that caprylic acid exhibited the strongest antihelminthic activity in comparison to other fatty acids and as a feed additive caprylic acid can be used as a safe and environment-friendly antiparasitic agent against *H. okamotoi*. It was also reported that caprylic acid exhibited a significantly stronger in vivo antiparasitic effect against *C. irritans* in *Pagrus major*, when compared to other medium-chain fatty acids (C_6–C_{10}) and short-chain fatty acids (C_2 and C_4) (Hirazawa et al. 2001b). Although specific mechanisms are not fully understood, caprylic acid has a broad-spectrum antimicrobial activity that might involve disruption of the bacterial cell membrane and intracellular acidification (Bergsson et al. 1998), disruption of specific enzymatic pathways (Brul and Coote 1999), or modification of the inter- and intracellular pH (Gauthier 2003). Emergence of antimicrobial-resistant strains of animal pathogens and their potential health risk to humans through foodborne transmission is a serious public health concern. Because of the extensive use of antibiotics, many gram-negative pathogens such as *Salmonella* spp. were found to acquire multiple drug-resistance genes (White et al. 2003). However, since fatty acids exert their antimicrobial effect by multiple mechanisms, the chances for bacterial resistance to caprylic acid are believed to be low.

2.3.4 Probiotics and Prebiotics

Probiotics The word probiotic in Greek means "for life," and the beneficial effects of probiotics have been widely reported (Gibson and Fuller 2000; Salminen et al. 2010; Salim et al. 2013; Serban 2014). A widely accepted definition of probiotics is "live microorganisms which when administered in adequate amounts can confer beneficial effects on host health" (Fuller 1989). The exact mechanisms by which probiotic bacteria confer health benefits are unclear; however, a wide range

of studies have identified potential mechanisms by which probiotic bacteria can elicit beneficial effects (Fuller 1989; Fooks et al. 1999; Salminen et al. 2010). Accordingly, probiotics may produce beneficial effects by improving barrier functions, by competing with pathogens for binding sites and nutrients, or by producing antimicrobial compounds (Fuller 1989; Sanders and Marco 2010; Serban 2014). Extensive research has been done, and several researchers have demonstrated the in vitro and in vivo efficacy of probiotic bacteria to inhibit enteric pathogens such as *Salmonella*, *Campylobacter*, and *E. coli* (Fooks and Gibson 2002; Chaveerach et al. 2004; Santini et al. 2010). Unfortunately, when trying to select and develop effective probiotic cultures, bacterial candidates with in vitro efficacy often fail to demonstrate similar efficacy in vivo (Santini et al. 2010; Robyn et al. 2012; Aguiar et al. 2013; Arsi et al. 2015a; Shrestha et al. 2017). This may be due to bacteria not being able to survive passage through the acidic stomach. Encapsulating these bacterial isolates may protect and allow passage into the lower intestine but does not guarantee their efficacy. In an attempt to demonstrate efficacy prior to the effort to encapsulate these isolates, Arsi et al. (2015b) administered bacterial isolates with in vitro efficacy intracloacally to evaluate their effectiveness in the lower GI tract. In addition, selecting bacteria that are GRAS (generally recognized as safe) will eliminate issues with undefined cultures and can be approved by the regulatory agencies for use in food animals. In a study comparing the oral versus intracloacal administration of probiotics, only one out of ten selected probiotic isolates given orally reduced *C. jejuni* populations by approximately 1 $\log_{10}$, whereas six out of ten isolates given intracloacally reduced *C. jejuni* populations by 1–3 $\log_{10}$ (Fig. 2.4, Arsi et al. 2015b), thus demonstrating that intracloacal administration of probiotics can be used to screen potential isolates against enteric pathogens and thereby overcome the inconsistencies associated with oral probiotics and pathogen colonization in broiler chickens. Another possible strategy for improving the efficacy of oral probiotics is to supplement dietary prebiotics along with the probiotic bacteria.

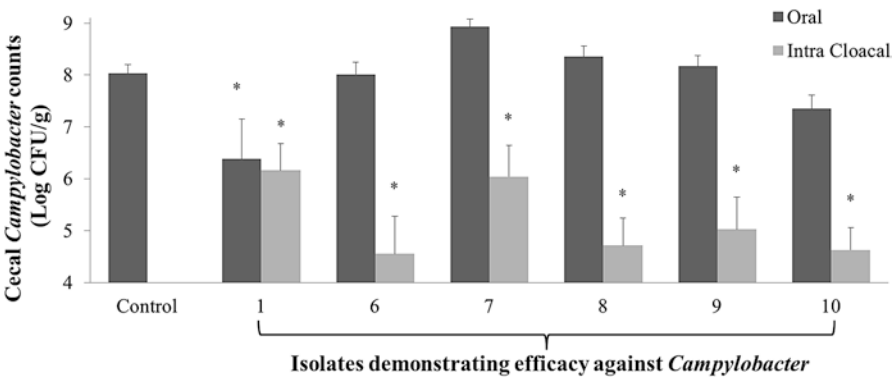

Fig. 2.4 Bacterial isolates demonstrating differences in *Campylobacter* colonization following either oral or intracloacal inoculation in 14-day-old broiler chickens. *Means which differ significantly from the control ($P < 0.05$). Reprinted with permission from Arsi et al. (2015b)

Prebiotics A prebiotic is defined as a "non-digestible food ingredient that beneficially affects the host by improving its intestinal microbial balance" (Gibson and Roberfroid 1995). While supplementing probiotics bring beneficial microflora to the gut, prebiotics can selectively enhance the growth of beneficial microflora and thereby protect the host from enteric pathogens (Patterson and Burkholder 2003; Macfarlane et al. 2006). There have been numerous studies on the benefits of inclusion of nondigestible carbohydrates to avian diets (Corrier et al. 1990a, b; DeLoach et al. 1990; Hinton et al. 1990; Waldroup 1993; McReynolds et al. 2007). One such carbohydrate is lactose, a natural disaccharide present in milk (composed of galactose and glucose). Researchers have shown that lactose can support gut health by promoting the growth of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* (Barrow 1992) and is capable of reducing enteric pathogens such as *Salmonella* (DeLoach et al. 1990; Corrier et al. 1990a, b; Hinton et al. 1990; Tellez et al. 1993; Nisbet et al. 1994), *Campylobacter* (Schoeni and Wong 1994), and *Clostridium* (McReynolds et al. 2007). Apart from lactose, other prebiotics that have been studied for their beneficial effects include inulin, fructo-oligosaccharides (FOS), mannan oligosaccharides (MOS), and galacto-oligosaccharides (GOS). Fructo-oligosaccharide supplementation in broiler diets improved the makeup of gut microflora by selectively supporting the growth of *Lactobacillus* spp. and inhibiting the pathogenic bacteria like *E. coli* and *Salmonella* (Xu et al. 2003). Similar efficacy was reported with mannan oligosaccharide (MOS), a prebiotic extracted from the yeast cell wall (Jamroz et al. 2004; Baurhoo et al. 2007a, b, 2009). Inulin is another naturally occurring polysaccharide that demonstrated a significant decrease in the enteric colonization of *E. coli*, *Salmonella*, and *Campylobacter* while selectively promoting the growth of *Bifidobacterium* spp. in the poultry GI tract (Rada et al. 2001; Yusrizal and Chen 2003; Velasco et al. 2010). Even though prebiotics alone can induce beneficial effects on host health, synbiotics (combination of probiotics and prebiotics) are proposed to be more efficacious than prebiotics or probiotics in producing the desired response (Serban 2014).

2.4 Other Challenges for Organic Poultry Producers

2.4.1 Availability of Organically Certified Feed Ingredients

According to the Organic Farming Research Foundation (OFRF), complying with the organic standards is one of the most pressing needs of organic livestock and poultry production (OFRF 2007). As per the National Organic Program rules (Federal Register 2017), all the ingredients used in the poultry feed should meet the organic standards. Currently, there is an imbalance between the demand and domestic supply of organic grains, and it is challenging to keep up with the growing demand and yet maintain the standards (NOC 2017). In general, no synthetics are allowed. Moreover, any ingredient used in organic poultry production should be certified organic. However, to supplement methionine, a limiting amino acid, synthetic DL-methionine, is temporarily allowed in organic poultry feed (NOC 2014).

This exemption is temporary, and organic producers continue to rely on this exemption to maintain their organic certification. A significant increase in dietary crude protein is needed to meet the methionine requirements, which is detrimental to bird health as well as the environment due to increased nitrogen excretion and ammonia emissions (van de Weerd et al. 2009; Burley et al. 2015). Unfortunately, there are limited alternatives that can supplement required quantities of methionine for the healthy growth and maintenance of organic poultry. Research is ongoing looking at a range of alternatives that can provide adequate levels of methionine in organic poultry rations (van de Weerd et al. 2009; Burley et al. 2015). Plant sources such as soybean meal, sesame meal, high methionine corn, naked oats, and Brazil nut meal and animal sources such as insect meal have been investigated with limited success. Fishmeal is a good source of methionine and is permitted in organic production. However, there are critical issues regarding cost, sustainable acquisition without depleting ocean stocks, and the use of synthetic preservatives. Currently we are evaluating Asian carp meal as a source of fish meal to supplement methionine for organic poultry. This invasive species has had a devastating impact in US waterways with no known predators (>$200 million/annually). These fish are boney, not normally eaten in the United States, and have little market value. Conservation of natural sources is a key part of ecological and organic production. If successful, Asian carp can be used as a sustainable source of methionine for organic poultry and also would reduce the impact of this invasive species on our waterways, thus, provide solutions to both of these issues.

2.4.2 Manure Management and Water

It is a common practice to use poultry manure as a soil amendment and for nutrient recycling in organic crop production. According to the NOP final rule, it is critically important for organic poultry producers to manage pathogens in manure to avoid any possible contamination to crops, soil, or water sources. It has to be noted that a 90–120-day interval should be followed between application of raw manure and harvest of crops that are intended for human consumption (USDA 2012). Organic poultry operations must comply with the regulations enforced by federal and state agencies to maintain organic certification. Apart from raising birds, organic poultry producers must also comply with manure-handling standards, take necessary steps to minimize soil erosion, and prevent contamination of water resources. Also, producers must ensure that the birds have access to clean drinking water at all times.

2.5 Conclusions

As organic poultry producers have a limited number of safe, effective, and approved organic strategies to prevent and treat foodborne pathogens in their flocks, effective solutions are necessary to provide safe organic products. Plant extracts,

probiotic/prebiotics, and organic acids have antimicrobial efficacy against poultry enteric pathogens and have potential in limiting contamination. These compounds are permitted under NOP and address food safety concerns in organic production systems. Further, these compounds/microbes potentially could also be used during postharvest processing to reduce the prevalence of foodborne pathogens on poultry products.

References

Aguiar, V. F., Donoghue, A. M., Arsi, K., Reyes-Herrera, I., Metcalf, J. H., de los Santos, F. S., et al. (2013). Targeting motility properties of bacteria in the development of probiotic cultures against *Campylobacter jejuni* in broiler chickens. *Foodborne Pathogens and Disease, 10*(5), 435–441.

Andreatti Filho, R. L., Silva, E. N. D., Ribeiro, A. R., Kondo, N., & Curi, P. R. (2000). Use of anaerobic cecal microflora, lactose and acetic acid for the protection of broiler chicks against experimental infection with *Salmonella* typhimurium and *Salmonella* enteritidis. *Brazilian Journal of Microbiology, 31*(2), 107–112.

Andrews, H. L., & Baumler, A. J. (2005). *Salmonella* species. In P. M. Fratamico, A. K. Bhunia, & J. L. Smith (Eds.), *Foodborne pathogens: Microbiology and molecular biology* (pp. 327–339). Norfolk, UK: Caister Academic Press.

Anonymous. (2011). *Six ill in Minnesota Salmonella egg outbreak*. Retrieved October 2017, from http://www.foodsafetynews.com/2011/10/six-ill-in-minnesota-salmonella-egg-outbreak/#.WH1CgVMrIUY

Anonymous. (2014). *Organic eggs recalled for Salmonella*. Retrieved October 2017, from https://foodpoisoningbulletin.com/2014/organic-eggs-recalled-for-salmonella/

Anonymous. (2016). *Good earth eggs recalled; linked to Salmonella outbreak*. Retrieved October 2017, from https://foodpoisoningbulletin.com/2016/good-earth-eggs-recalled-linked-to-salmonella-outbreak/

Arsi, K., Donoghue, A. M., Venkitanarayanan, K., Kollanoor-Johny, A., Fanatico, A. C., Blore, P. J., et al. (2014). The efficacy of the natural plant extracts, thymol and carvacrol against *Campylobacter* colonization in broiler chickens. *Journal of Food Safety, 34*(4), 321–325.

Arsi, K., Donoghue, A. M., Woo-Ming, A., Blore, P. J., & Donoghue, D. J. (2015a). The efficacy of selected probiotic and prebiotic combinations in reducing *Campylobacter* colonization in broiler chickens. *Journal of Applied Poultry Research, 24*(3), 327–334.

Arsi, K., Donoghue, A. M., Woo-Ming, A., Blore, P. J., & Donoghue, D. J. (2015b). Intracloacal inoculation, an effective screening method for determining the efficacy of probiotic bacterial isolates against *Campylobacter* colonization in broiler chickens. *Journal of Food Protection, 78*(1), 209–213.

Arsi, K., Donoghue, A. M., Upadhyaya, I., Upadhyay, A., Wagle, B. R., Shrestha, S., et al. (2017). Alternatives to antibiotics: Novel strategies to reduce foodborne pathogens in organic poultry. In: *Proceedings of the Midwest poultry federation convention.*

Ayoola, G. A., Lawore, F. M., Adelowotan, T., Aibinu, I. E., Adenipekun, E., Coker, H. A. B., et al. (2008). Chemical analysis and antimicrobial activity of the essential oil of *Syzygium aromaticum* (clove). *African Journal of Microbiology Research, 2*(7), 162–166.

Bailey, J. S., & Cosby, D. E. (2005). *Salmonella* prevalence in free-range and certified organic chickens. *Journal of Food Protection, 68*(11), 2451–2453.

Barrow, P. A. (1992). Probiotics for chickens. In R. Fuller (Ed.), *Probiotics: The scientific basis* (pp. 225–257). London: Chapman and Hall.

Baskaran, S. A., Upadhyay, A., Kollanoor-Johny, A., Upadhyaya, I., Mooyottu, S., Roshni Amalaradjou, M. A., et al. (2013). Efficacy of plant-derived antimicrobials as antimicrobial wash treatments for reducing enterohemorrhagic *Escherichia Coli* O157:H7 on apples. *Journal of Food Science, 78*(9), M1399.

Baskaran, S. A., Kollanoor-Johny, A., Nair, M. S., & Venkitanarayanan, K. (2016). Efficacy of plant-derived antimicrobials in controlling enterohemorrhagic *Escherichia coli* virulence *in vitro*. *Journal of Food Protection, 79*(11), 1965–1970.

Baurhoo, B., Letellier, A., Zhao, X., & Ruiz-Feria, C. A. (2007a). Cecal populations of *Lactobacilli* and *Bifidobacteria* and *Escherichia coli* populations after *in vivo Escherichia coli* challenge in birds fed diets with purified lignin or mannanoligosaccharides. *Poultry Science, 86*(12), 2509–2516.

Baurhoo, B., Phillip, L., & Ruiz-Feria, C. A. (2007b). Effects of purified lignin and mannan oligosaccharides on intestinal integrity and microbial populations in the ceca and litter of broiler chickens. *Poultry Science, 86*(6), 1070–1078.

Baurhoo, B., Ferket, P. R., & Zhao, X. (2009). Effects of diets containing different concentrations of mannanoligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers. *Poultry Science, 88*(11), 2262–2272.

Beretz, A., Anton, R., & Stoclet, J. C. (1978). Flavonoid compounds are potent inhibitors of cyclic AMP phosphodiesterase. *Cellular and Molecular Life Sciences, 34*(8), 1054–1055.

Berg, C. (2002). Health and welfare in organic poultry production. *Acta Veterinaria Scandinavica, 43*(1), S37.

Bergsson, G., Arnfinnsson, J., Karlsson, S. M., Steingrímsson, Ó., & Thormar, H. (1998). *In vitro* inactivation of *Chlamydia trachomatis* by fatty acids and monoglycerides. *Antimicrobial Agents and Chemotherapy, 42*(9), 2290–2294.

Blaszyk, M., & Holley, R. A. (1998). Interaction of monolaurin, eugenol and sodium citrate on growth of common meat spoilage and pathogenic organisms. *International Journal of Food Microbiology, 39*(3), 175–183.

Bowles, B. L., & Miller, A. J. (1993). Antibotulinal properties of selected aromatic and aliphatic ketones. *Journal of Food Protection, 56*(9), 795–800.

Brown, L. G., Khargonekar, S., Bushnell, L., & Environmental Health Specialists Network Working Group. (2013). Frequency of inadequate chicken cross-contamination prevention and cooking practices in restaurants. *Journal of Food Protection, 76*(12), 2141–2145.

Brul, S., & Coote, P. (1999). Preservative agents in foods: Mode of action and microbial resistance mechanisms. *International Journal of Food Microbiology, 50*(1), 1–17.

Burley, H. K., Patterson, P. H., & Anderson, K. E. (2015). Alternative ingredients for providing adequate methionine in organic poultry diets in the United States with limited synthetic amino acid use. *World's Poultry Science Journal, 71*(3), 493–504.

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods—A review. *International Journal of Food Microbiology, 94*(3), 223–253.

CDC. (2015). *Centers for Disease Control and Prevention. Food safety – Foodborne germs and illnesses*. Retrieved October 2017, from https://www.cdc.gov/foodsafety/foodborne-germs.html

CDC. (2017a). *Centers for Disease Control and Prevention. Campylobacter, Salmonella led bacterial foodborne illnesses in 2016*. Retrieved October 2017, from https://www.cdc.gov/media/releases/2017/p0420-campylobacter-salmonella.html

CDC. (2017b). *Centers for Disease Control and Prevention. Salmonella: Multistate outbreaks of human Salmonella infections linked to live poultry in backyard flocks*, 2017. Retrieved October 2017, from https://www.cdc.gov/salmonella/live-poultry-06-17/index.html

Chaveerach, P., Keuzenkamp, D. A., Urlings, H. A., Lipman, L. J., & Van Knapen, F. (2002). *In vitro* study on the effect of organic acids on *Campylobacter jejuni/coli* populations in mixtures of water and feed. *Poultry Science, 81*(5), 621–628.

Chaveerach, P., Lipman, L. J. A., & Van Knapen, F. (2004). Antagonistic activities of several bacteria on *in vitro* growth of 10 strains of *Campylobacter jejuni/coli*. *International Journal of Food Microbiology, 90*(1), 43–50.

Cobanoglu, F., Kucukyilmaz, K., Cinar, M., Bozkurt, M., Catli, A. U., & Bintas, E. (2014). Comparing the profitability of organic and conventional broiler production. *Revista Brasileira de Ciência Avícola, 16*(1), 89–95.

Corrier, D. E., Hinton Jr., A., Ziprin, R. L., & DeLoach, J. R. (1990a). Effect of dietary lactose on *Salmonella* colonization of market-age broiler chickens. *Avian Diseases, 34*, 668–676.

Corrier, D. E., Hinton Jr., A., Ziprin, R. L., Beier, R. C., & DeLoach, J. R. (1990b). Effect of dietary lactose on cecal pH, bacteriostatic volatile fatty acids, and *Salmonella typhimurium* colonization of broiler chicks. *Avian Diseases, 34*, 617–625.

Cox, S. D., & Markham, J. L. (2007). Susceptibility and intrinsic tolerance of *Pseudomonas aeruginosa* to selected plant volatile compounds. *Journal of Applied Microbiology, 103*(4), 930–936.

Cui, S., Ge, B., Zheng, J., & Meng, J. (2005). Prevalence and antimicrobial resistance of Campylobacter spp. and Salmonella serovars in organic chickens from Maryland retail stores. *Applied and Environmental Microbiology, 71*(7), 4108–4111.

Darre, M. J., Kollanoor-Johny, A., Venkitanarayanan, K., & Upadhyaya, I. (2014). Practical implications of plant-derived antimicrobials in poultry diets for the control of *Salmonella* enteritidis. *Journal of Applied Poultry Research, 23*(2), 340–344.

DeLoach, J. R., Oyofo, B. A., Corrier, D. E., Kubena, L. F., Ziprin, R. L., & Norman, J. O. (1990). Reduction of *Salmonella* typhimurium concentration in broiler chickens by milk or whey. *Avian Diseases, 34*, 389–392.

Donato, F., & Zani, C. (2010). Chronic exposure to organochlorine compounds and health effects in adults: Cancer, non-Hodgkin lymphoma. Review of literature. *Annali di Igiene: Medicina Preventiva e di Comunita, 22*(4), 357–367.

Dore, M. H. (2015). Threats to human health: Use of chlorine, an obsolete treatment technology. In *Global drinking water management and conservation* (pp. 197–212). Cham, Switzerland: Springer.

Dorman, H. J. D., & Deans, S. G. (2000). Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *Journal of Applied Microbiology, 88*(2), 308–316.

Engvall, A. (2002). May organically farmed animals pose a risk for *Campylobacter* infections in humans? *Acta Veterinaria Scandinavica, 43*(1), S85.

Fanatico, A. (2006). Organic poultry production in the United States. *ATTRA. NCAT*. Retrieved October 2017, from www.attra.ncat.org/attra-pub/pPDF/organicpoultry.pdf

Fanatico, A. C., Owens, C. M., & Emmert, J. L. (2009). Organic poultry production in the United States: Broilers. *Journal of Applied Poultry Research, 18*(2), 355–366.

FDA. (2011). *Salmonella illnesses linked to organic eggs consumers, food preparers reminded to cook eggs thoroughly.* Retrieved October 2017, from http://www.fda.gov/Safety/Recalls/ucm276901.htm

FDA. (2013). *U.S. Food and Drug Administration: Everything added to food in the United States (EAFUS)*. Retrieved October 2017, from https://www.accessdata.fda.gov/scripts/fcn/fcnNavigation.cfm?filter=89-86-1&sortColumn=&rpt=eafusListing

FDA. (2016). *Good earth egg company voluntarily recalls shell eggs because of a possible health risk*. Retrieved October 2017, from http://www.fda.gov/Safety/Recalls/ucm523727.htm?source=govdelivery&utm_medium=email&utm_source=govdelivery

FDA. (2017). *U.S. Food and Drug Administration: CFR - Code of Federal Regulations Title 21*. Retrieved October 2017, from https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=182.20

Federal Register. (2017). *National Organic Program (NOP); Organic livestock and poultry practices*. Retrieved October 2017, from https://www.gpo.gov/fdsys/pkg/FR-2017-01-19/pdf/2017-00888.pdf

Fooks, L. J., & Gibson, G. R. (2002). Probiotics as modulators of the gut flora. *British Journal of Nutrition, 88*(S1), s39–s49.

Fooks, L. J., Fuller, R., & Gibson, G. R. (1999). Prebiotics, probiotics and human gut microbiology. *International Dairy Journal, 9*(1), 53–61.

Friedman, M. (2014). Chemistry and multibeneficial bioactivities of carvacrol (4-isopropyl-2-methylphenol), a component of essential oils produced by aromatic plants and spices. *Journal of Agricultural and Food Chemistry, 62*(31), 7652–7670.

Friedman, M., Henika, P. R., & Mandrell, R. E. (2003). Antibacterial activities of phenolic benzaldehydes and benzoic acids against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *Journal of Food Protection, 66*(10), 1811–1821.

Friedman, M., Buick, R., & Elliott, C. T. (2004). Antibacterial activities of naturally occurring compounds against antibiotic-resistant *Bacillus cereus* vegetative cells and spores, *Escherichia coli*, and *Staphylococcus aureus*. *Journal of Food Protection, 67*(8), 1774–1778.

FSIS. (2015). *Draft FSIS compliance guideline for controlling Salmonella and Campylobacter in raw poultry*. Retrieved November 2017, from https://www.fsis.usda.gov/wps/wcm/connect/6732c082-af40-415e-9b57-90533ea4c252/Controlling-Salmonella-Campylobacter-Poultry-2015.pdf?MOD=AJPERES

Fuller, R. (1989). Probiotics in man and animals. *The Journal of Applied Bacteriology, 66*(5), 365–378.

Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Gast, R., Humphrey, T. J., et al. (2009). Mechanisms of egg contamination by *Salmonella* enteritidis. *FEMS Microbiology Reviews, 33*(4), 718–738.

Gast, R. K., & Beard, C. W. (1990). Production of *Salmonella* enteritidis-contaminated eggs by experimentally infected hens. *Avian Diseases, 34*, 438–446.

Gauthier, R. (2003). Poultry therapeutics: New alternatives in nuevas alternativas en therapeutica aviar. In *XVIII Latin American poultry congress 2003, Bolivia*.

Geissman, T. A. (1963). Flavonoid compounds, tannins, lignins and related compounds. In M. Florkin & E. H. Stotz (Eds.), *Pyrrole pigments, isoprenoid compounds and phenolic plant constituents* (Vol. 9, p. 265). Amsterdam: Elsevier.

Gibson, G. R., & Fuller, R. (2000). Aspects of *in vitro* and *in vivo* research approaches directed toward identifying probiotics and prebiotics for human use. *The Journal of Nutrition, 130*(2), 391S–395S.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *The Journal of Nutrition, 125*(6), 1401.

Gill, A. O., & Holley, R. A. (2004). Mechanisms of bactericidal action of cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus sakei*. *Applied and Environmental Microbiology, 70*(10), 5750–5755.

Glos, K. (2011). *Humane and healthy poultry production: A manual for organic growers*. White River Junction, VT: Chelsea Green Publishing.

Hargis, B. M., Caldwell, D. J., Byrd, J. A., Corrier, D. E., & Stanker, L. H. (1998). Preharvest crop contamination and *Salmonella* recovery from broiler carcasses at processing. In *Proceedings, international symposium on food-borne Salmonella in poultry, Baltimore, MD, July 25–26, 1998* (pp. 220–231). American Association of Avian Pathologists.

Harper, G. C., & Makatouni, A. (2002). Consumer perception of organic food production and farm animal welfare. *British Food Journal, 104*(3/4/5), 287–299.

Hinton Jr., A., Corrier, D. E., Spates, G. E., Norman, J. O., Ziprin, R. L., Beier, R. C., et al. (1990). Biological control of *Salmonella typhimurium* in young chickens. *Avian Diseases, 34*, 626–633.

Hirazawa, N., Oshima, S. I., & Hata, K. (2001a). *In vitro* assessment of the antiparasitic effect of caprylic acid against several fish parasites. *Aquaculture, 200*(3), 251–258.

Hirazawa, N., Oshima, S. I., Hara, T., Mitsuboshi, T., & Hata, K. (2001b). Antiparasitic effect of medium-chain fatty acids against the ciliate *Cryptocaryon irritans* infestation in the red sea bream *Pagrus major*. *Aquaculture, 198*(3), 219–228.

IFOAM. (2009). *IFOAM basic standards*. International Federation of Organic Movements, Tholey-theley. Retrieved October 2017, from www.ifoam.org/standard/basics

Jacob, M. E., Fox, J. T., Reinstein, S. L., & Nagaraja, T. G. (2008). Antimicrobial susceptibility of foodborne pathogens in organic or natural production systems: An overview. *Foodborne Pathogens and Disease, 5*(6), 721–730.

Jamroz, D., Wiliczkiewicz, A., Orda, J., Wertelecki, T., & Skorupinska, J. (2004). Response of broiler chickens to the diets supplemented with feeding antibiotic or mannanoligosaccharides. *Electronic Journal of Polish Agricultural Universities Series Animal Husbandry, 7*(2), 1–6.

Jensen, R. G. (2002). The composition of bovine milk lipids: January 1995 to December 2000. *Journal of Dairy Science, 85*(2), 295–350.

Johny, A. K., Darre, M. J., Hoagland, T. A., Schreiber, D. T., Donoghue, A. M., Donoghue, D. J., et al. (2008). Antibacterial effect of trans-cinnamaldehyde on *Salmonella* enteritidis and *Campylobacter jejuni* in chicken drinking water. *Journal of Applied Poultry Research, 17*(4), 490–497.

Johny, A. K., Baskaran, S. A., Charles, A. S., Amalaradjou, M. A. R., Darre, M. J., Khan, M. I., et al. (2009). Prophylactic supplementation of caprylic acid in feed reduces *Salmonella* enteritidis colonization in commercial broiler chicks. *Journal of Food Protection, 72*(4), 722–727.

Kijlstra, A., & Eijck, I. A. J. M. (2006). Animal health in organic livestock production systems: A review. *NJAS - Wageningen Journal of Life Sciences, 54*(1), 77–94.

Kollanoor Johny, A., Darre, M. J., Donoghue, A. M., Donoghue, D. J., & Venkitanarayanan, K. (2010). Antibacterial effect of trans-cinnamaldehyde, eugenol, carvacrol, and thymol on *Salmonella* enteritidis and *Campylobacter jejuni* in chicken cecal contents *in vitro*. *Journal of Applied Poultry Research, 19*(3), 237–244.

Kollanoor Johny, A., Mattson, T., Baskaran, S. A., Amalaradjou, M. A., Babapoor, S., March, B., et al. (2012). Reduction of *Salmonella enterica* serovar enteritidis colonization in 20-day-old broiler chickens by the plant-derived compounds trans-cinnamaldehyde and eugenol. *Applied and Environmental Microbiology, 78*(8), 2981–2987.

Lin, J. (2009). Novel approaches for *Campylobacter* control in poultry. *Foodborne Pathogens and Disease, 6*(7), 755–765.

Loretz, M., Stephan, R., & Zweifel, C. (2010). Antimicrobial activity of decontamination treatments for poultry carcasses: A literature survey. *Food Control, 21*(6), 791–804.

Lund, V. (2006). Natural living—A precondition for animal welfare in organic farming. *Livestock Science, 100*(2), 71–83.

Lund, V., & Algers, B. (2003). Research on animal health and welfare in organic farming—A literature review. *Livestock Production Science, 80*(1), 55–68.

Macfarlane, S. M. G. T., Macfarlane, G. T., & Cummings, J. T. (2006). Prebiotics in the gastrointestinal tract. *Alimentary Pharmacology & Therapeutics, 24*(5), 701–714.

Magkos, F., Arvaniti, F., & Zampelas, A. (2003). Putting the safety of organic food into perspective. *Nutrition Research Reviews, 16*(2), 211–222.

Marchese, A., Orhan, I. E., Daglia, M., Barbieri, R., Di Lorenzo, A., Nabavi, S. F., et al. (2016). Antibacterial and antifungal activities of thymol: A brief review of the literature. *Food Chemistry, 210*, 402–414.

Mattson, T. E., Johny, A. K., Amalaradjou, M. A. R., More, K., Schreiber, D. T., Patel, J., et al. (2011). Inactivation of *Salmonella* spp. on tomatoes by plant molecules. *International Journal of Food Microbiology, 144*(3), 464–468.

McReynolds, J. L., Byrd, J. A., Genovese, K. J., Poole, T. L., Duke, S. E., Farnell, M. B., et al. (2007). Dietary lactose and its effect on the disease condition of necrotic enteritis. *Poultry Science, 86*(8), 1656–1661.

Messens, W., Grijspeerdt, K., & Herman, L. (2005). Eggshell penetration by *Salmonella*: A review. *World's Poultry Science Journal, 61*(1), 71–86.

Messens, W., Grijspeerdt, K., & Herman, L. (2006). Eggshell penetration of hen's eggs by *Salmonella enterica* serovar enteritidis upon various storage conditions. *British Poultry Science, 47*(5), 554–560.

Mitsch, P., Zitterl-Eglseer, K., Köhler, B., Gabler, C., Losa, R., & Zimpernik, I. (2004). The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens* in the intestines of broiler chickens. *Poultry Science, 83*(4), 669–675.

Nachamkin, I., & Guerry, P. (2005). *Campylobacter* infections. In P. M. Fratamico, A. K. Bhunia, & J. L. Smith (Eds.), *Foodborne pathogens: Microbiology and molecular biology* (pp. 285–293). Norfolk, UK: Caister Academic Press.

Nair, M. K. M., Joy, J., Vasudevan, P., Hinckley, L., Hoagland, T. A., & Venkitanarayanan, K. S. (2005). Antibacterial effect of caprylic acid and monocaprylin on major bacterial mastitis pathogens. *Journal of Dairy Science, 88*(10), 3488–3495.

Nisbet, D. J., Corrier, D. E., Scanlan, C. M., Hollister, A. G., Beier, R. C., & Deloach, J. R. (1994). Effect of dietary lactose and cell concentration on the ability of a continuous-flow-derived bacterial culture to control *Salmonella* cecal colonization in broiler chickens. *Poultry Science, 73*(1), 56–62.

NOC. (2014). *National Organic Coalition. Comments to the livestock sub-committee*. Retrieved October 2017, from http://www.nationalorganiccoalition.org/_literature_122844/NOC_NOSB_Comments-Livestock_Sub-committee-April_8,_2014

NOC. (2017). *National Organic Coalition. Expanding organic production in the United States: Challenges & policy recommendations*. Retrieved October 2017, from http://www.nationalorganiccoalition.org/LiteratureRetrieve.aspx?ID=135718

NOP. (2009). *National Organic Program. The National List of Allowed and Prohibited Substances*. Retrieved October 2017, from http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5068682&acct=nopgeninfo

Novak, J. S., Peck, M. W., Juneja, V. K., & Johnson, E. A. (2005). *Clostridium botulinum* and *Clostridium perfringens*. In P. M. Fratamico, A. K. Bhunia, & J. L. Smith (Eds.), *Foodborne pathogens: Microbiology and molecular biology* (Vol. 38, pp. 383–407). Norfolk, UK: Caister Academic Press.

Noyes, J. (2009). *Northern and Central California eggs recall*. Retrieved October 2017, from http://www.nbclosangeles.com/news/local/Northern-California-Egg-Recall-Announced.html

OACC. (2008). *Organic Agriculture Centre of Canada. Research needs assessment of British Columbia organic farmers*. Truro, NS: Nova Scotia Agricultural College. Retrieved October 2017, from https://cdn.dal.ca/content/dam/dalhousie/pdf/faculty/agriculture/oacc/en/research-priorities/Canadian_Organic_Research_Needs_Survey_BC_2008.pdf

OFRF. (2007). *Organic Farming Research Foundation. National Organic Research Agenda: Soils, pests, livestock, genetics. Outcomes from the scientific congress on Organic Agricultural Research (SCOAR)*. Retrieved October 2017, from http://ofrf.org/sites/ofrf.org/files/nora2007.pdf

Oscar, T. P. (2013). Initial contamination of chicken parts with *Salmonella* at retail and cross-contamination of cooked chicken with *Salmonella* from raw chicken during meal preparation. *Journal of Food Protection, 76*(1), 33–39.

OTA. (2017). *Organic Trade Association: Robust organic sector stays on upward climb, posts new records in U.S. sales*. Retrieved October 2017, from https://www.ota.com/news/press-releases/19681

Padgham, J. (2006). Introduction to pastured poultry. In *Raising poultry on pasture: Ten years of success*. Hughesville, PA: The American Pastured Poultry Producers Association Compilation.

Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V., Braden, C. R., Angulo, F. J., et al. (2013). Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerging Infectious Diseases, 19*(3), 407–415.

Palaniappan, K., & Holley, R. A. (2010). Use of natural antimicrobials to increase antibiotic susceptibility of drug resistant bacteria. *International Journal of Food Microbiology, 140*(2), 164–168.

PAN. (2007). *Pesticides database – California pesticide use*. Retrieved October 2017, from http://www.pesticideinfo.org/Detail_ChemUse.jsp

Patterson, J. A., & Burkholder, K. M. (2003). Application of prebiotics and probiotics in poultry production. *Poultry Science, 82*(4), 627–631.

Petschow, B. W., Batema, R. P., & Ford, L. L. (1996). Susceptibility of *Helicobacter pylori* to bactericidal properties of medium-chain monoglycerides and free fatty acids. *Antimicrobial Agents and Chemotherapy, 40*(2), 302–306.

Prabuseenivasan, S., Jayakumar, M., & Ignacimuthu, S. (2006). *In vitro* antibacterial activity of some plant essential oils. *BMC Complementary and Alternative Medicine, 6*(1), 39.

Rada, V., Dušková, D., Marounek, M., & Petr, J. (2001). Enrichment of Bifidobacteria in the hen caeca by dietary inulin. *Folia Microbiologica, 46*(1), 73–75.

Ravishankar, S., Zhu, L., Law, B., Joens, L., & Friedman, M. (2008). Plant-derived compounds inactivate antibiotic-resistant *Campylobacter jejuni* strains. *Journal of Food Protection, 71*(6), 1145–1149.

Richardson, S. D., Thruston, A. D., Caughran, T. V., Collette, T. W., Patterson, K. S., & Lykins, B. W. (1998). Chemical by-products of chlorine and alternative disinfectants. *Food Technology, 52*(4), 58–61.

Robyn, J., Rasschaert, G., Messens, W., Pasmans, F., & Heyndrickx, M. (2012). Screening for lactic acid bacteria capable of inhibiting *Campylobacter jejuni* in *in vitro* simulations of the broiler chicken caecal environment. *Beneficial Microbes, 3*(4), 299–308.

Rosenquist, H., Nielsen, N. L., Sommer, H. M., Nørrung, B., & Christensen, B. B. (2003). Quantitative risk assessment of human campylobacteriosis associated with thermophilic *Campylobacter* species in chickens. *International Journal of Food Microbiology, 83*(1), 87–103.

Salim, H. M., Kang, H. K., Akter, N., Kim, D. W., Kim, J. H., Kim, M. J., et al. (2013). Supplementation of direct-fed microbials as an alternative to antibiotic on growth performance, immune response, cecal microbial population, and ileal morphology of broiler chickens. *Poultry Science, 92*(8), 2084–2090.

Salminen, S., Nybom, S., Meriluoto, J., Collado, M. C., Vesterlund, S., & El-Nezami, H. (2010). Interaction of probiotics and pathogens—Benefits to human health? *Current Opinion in Biotechnology, 21*(2), 157–167.

Sanders, M. E., & Marco, M. L. (2010). Food formats for effective delivery of probiotics. *Annual Review of Food Science and Technology, 1*, 65–85.

Santini, C., Baffoni, L., Gaggia, F., Granata, M., Gasbarri, R., Di Gioia, D., et al. (2010). Characterization of probiotic strains: An application as feed additives in poultry against *Campylobacter jejuni*. *International Journal of Food Microbiology, 141*, S98–S108.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., et al. (2011). Foodborne illness acquired in the United States—Major pathogens. *Emerging Infectious Diseases, 17*(1), 7.

Schoeni, J. L., & Wong, A. C. (1994). Inhibition of *Campylobacter jejuni* colonization in chicks by defined competitive exclusion bacteria. *Applied and Environmental Microbiology, 60*(4), 1191–1197.

Serban, D. E. (2014). Gastrointestinal cancers: Influence of gut microbiota, probiotics and prebiotics. *Cancer Letters, 345*(2), 258–270.

Shahverdi, A. R., Monsef-Esfahani, H. R., Tavasoli, F., Zaheri, A., & Mirjani, R. (2007). Transcinnamaldehyde from Cinnamomum zeylanicum bark essential oil reduces the clindamycin resistance of *Clostridium difficile in vitro*. *Journal of Food Science, 72*(1), 55–58.

Shen, S., Zhang, T., Yuan, Y., Lin, S., Xu, J., & Ye, H. (2015). Effects of cinnamaldehyde on *Escherichia coli* and *Staphylococcus aureus* membrane. *Food Control, 47*, 196–202.

Shrestha, S., Arsi, K., Wagle, B., Donoghue, A., & Donoghue, D. (2017). Ability of select probiotics to reduce enteric *Campylobacter* colonization in broiler chickens. *International Journal of Poultry Science, 16*, 37–42.

Si, W., Gong, J., Tsao, R., Zhou, T., Yu, H., Poppe, C., et al. (2006). Antimicrobial activity of essential oils and structurally related synthetic food additives towards selected pathogenic and beneficial gut bacteria. *Journal of Applied Microbiology, 100*(2), 296–305.

Si, W., Ni, X., Gong, J., Yu, H., Tsao, R., Han, Y., et al. (2009). Antimicrobial activity of essential oils and structurally related synthetic food additives towards *Clostridium perfringens*. *Journal of Applied Microbiology, 106*(1), 213–220.

Skřivanová, E., Marounek, M., Benda, V., & Březina, P. (2006). Susceptibility of *Escherichia coli*, *Salmonella* sp. and *Clostridium perfringens* to organic acids and monolaurin. *Veterinární Medicína, 51*(3), 81–88.

Solis de Los Santos, F., Donoghue, A. M., Venkitanarayanan, K., Dirain, M. L., Reyes-Herrera, I., Blore, P. J., et al. (2008). Caprylic acid supplemented in feed reduces enteric *Campylobacter jejuni* colonization in ten-day-old broiler chickens. *Poultry Science, 87*(4), 800–804.

Solis de Los Santos, F., Donoghue, A. M., Venkitanarayanan, K., Metcalf, J. H., Reyes-Herrera, I., Dirain, M. L., et al. (2009). The natural feed additive caprylic acid decreases *Campylobacter jejuni* colonization in market-aged broiler chickens. *Poultry Science, 88*(1), 61–64.

Solis de Los Santos, F., Hume, M., Venkitanarayanan, K., Donoghue, A. M., Hanning, I., Slavik, M. F., et al. (2010). Caprylic acid reduces enteric *Campylobacter* colonization in market-aged broiler chickens but does not appear to alter cecal microbial populations. *Journal of Food Protection, 73*(2), 251–257.

Sprong, R. C., Hulstein, M. F., & Van der Meer, R. (2001). Bactericidal activities of milk lipids. *Antimicrobial Agents and Chemotherapy, 45*(4), 1298–1301.

Stern, N. J. (2008). *Salmonella* species and *Campylobacter jejuni* cecal colonization model in broilers. *Poultry Science, 87*(11), 2399–2403.

Sundrum, A. (2001). Organic livestock farming: A critical review. *Livestock Production Science, 67*(3), 207–215.

Taylor, M., Joerger, R., Palou, E., López-Malo, A., Avila-Sosa, R., & Calix-Lara, T. (2012). Alternatives to traditional antimicrobials for organically processed meat and poultry. In S. C. Ricke, E. J. Van Loo, M. G. Johnson, & C. A. O'Bryan (Eds.), *Organic meat production and processing* (pp. 211–230). Hoboken, NJ: Wiley.

Tellez, G., Dean, C. E., Corrier, D. E., Deloach, J. R., Jaeger, L., & Hargis, B. M. (1993). Effect of dietary lactose on cecal morphology, pH, organic acids, and *Salmonella* enteritidis organ invasion in Leghorn chicks. *Poultry Science, 72*(4), 636–642.

Thormar, H., Hilmarsson, H., & Bergsson, G. (2006). Stable concentrated emulsions of the 1-monoglyceride of capric acid (monocaprin) with microbicidal activities against the food-borne bacteria *Campylobacter jejuni*, *Salmonella* spp., and *Escherichia coli*. *Applied and Environmental Microbiology, 72*(1), 522–526.

Tuyttens, F., Heyndrickx, M., De Boeck, M., Moreels, A., Van Nuffel, A., Van Poucke, E., et al. (2008). Broiler chicken health, welfare and fluctuating asymmetry in organic versus conventional production systems. *Livestock Science, 113*(2), 123–132.

Upadhyay, A., Johny, A. K., Amalaradjou, M. A. R., Baskaran, S. A., Kim, K. S., & Venkitanarayanan, K. (2012). Plant-derived antimicrobials reduce *Listeria monocytogenes* virulence factors *in vitro*, and down-regulate expression of virulence genes. *International Journal of Food Microbiology, 157*(1), 88–94.

Upadhyay, A., Upadhyaya, I., Kollanoor-Johny, A., & Venkitanarayanan, K. (2013). Antibiofilm effect of plant derived antimicrobials on *Listeria monocytogenes*. *Food Microbiology, 36*(1), 79–89.

Upadhyaya, I., Upadhyay, A., Kollanoor-Johny, A., Baskaran, S. A., Mooyottu, S., Darre, M. J., et al. (2013). Rapid inactivation of *Salmonella* enteritidis on shell eggs by plant-derived antimicrobials. *Poultry Science, 92*(12), 3228–3235.

Upadhyaya, I., Upadhyay, A., Kollanoor-Johny, A., Mooyottu, S., Baskaran, S. A., Yin, H. B., et al. (2015). In-feed supplementation of trans-cinnamaldehyde reduces layer-chicken egg-borne transmission of *Salmonella* enterica serovar enteritidis. *Applied and Environmental Microbiology, 81*(9), 2985–2994.

Upadhyaya, I., Fancher, S., Yin, H., Nair, M. S., Chen, C., Karumathil, D., et al. (2016). Reducing *Salmonella* Heidelberg colonization in 21-day-old broiler chicks by in-feed supplementation of β-resorcylic acid and trans-cinnamaldehyde. In *2016 PSA Annual Meeting, Poultry Science, 95*(E-suppl. 1), 162.

USDA. (2012). *Guide for organic crop producers*. Retrieved November 2017, from https://www.ams.usda.gov/sites/default/files/media/GuideForOrganicCropProducers.pdf

van de Weerd, H. A., Keatinge, R., & Roderick, S. (2009). A review of key health-related welfare issues in organic poultry production. *World's Poultry Science Journal, 65*(4), 649–684.

van Immerseel, F., De Buck, J., Boyen, F., Bohez, L., Pasmans, F., Volf, J., et al. (2004a). Medium-chain fatty acids decrease colonization and invasion through hilA suppression shortly after infection of chickens with *Salmonella enterica* serovar Enteritidis. *Applied and Environmental Microbiology, 70*(6), 3582–3587.

van Immerseel, F., Buck, J. D., Pasmans, F., Huyghebaert, G., Haesebrouck, F., & Ducatelle, R. (2004b). *Clostridium perfringens* in poultry: An emerging threat for animal and public health. *Avian Pathology, 33*(6), 537–549.

Van Overbeke, I., Duchateau, L., De Zutter, L., Albers, G., & Ducatelle, R. (2006). A comparison survey of organic and conventional broiler chickens for infectious agents affecting health and food safety. *Avian Diseases, 50*, 196–200.

Vasudevan, P., Marek, P., Nair, M. K. M., Annamalai, T., Darre, M., Khan, M., et al. (2005). *In vitro* inactivation of *Salmonella* enteritidis in autoclaved chicken cecal contents by caprylic acid. *Journal of Applied Poultry Research, 14*(1), 122–125.

Velasco, S., Ortiz, L. T., Alzueta, C., Rebole, A., Trevino, J., & Rodriguez, M. L. (2010). Effect of inulin supplementation and dietary fat source on performance, blood serum metabolites, liver lipids, abdominal fat deposition, and tissue fatty acid composition in broiler chickens. *Poultry Science, 89*(8), 1651–1662.

Wagle, B. R., Upadhyay, A., Arsi, K., Shrestha, S., Venkitanarayanan, K., Donoghue, A. M., et al. (2017a). Application of β-resorcylic acid as potential antimicrobial feed additive to reduce *Campylobacter* colonization in broiler chickens. *Frontiers in Microbiology, 8*, 599.

Wagle, B. R., Arsi, K., Upadhyay, A., Shrestha, S., Venkitanarayanan, K., Donoghue, A. M., et al. (2017b). β-resorcylic acid, a phytophenolic compound, reduces *Campylobacter jejuni* in post-harvest poultry. *Journal of Food Protection, 80*(8), 1243–1251.

Waldroup, A. L. (1993). Summary of work to control pathogens in poultry processing. *Poultry Science, 72*(6), 1177–1179.

White, D. G., Datta, A., McDermott, P., Friedman, S., Qaiyumi, S., Ayers, S., et al. (2003). Antimicrobial susceptibility and genetic relatedness of *Salmonella* serovars isolated from animal-derived dog treats in the USA. *Journal of Antimicrobial Chemotherapy, 52*(5), 860–863.

Wollenweber, E. (1988). Occurrence of flavonoid aglycones in medicinal plants. *Progress in Clinical and Biological Research, 280*, 45–55.

Xu, Z. R., Hu, C. H., Xia, M. S., Zhan, X. A., & Wang, M. Q. (2003). Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poultry Science, 82*(6), 1030–1036.

Xu, J., Zhou, F., Ji, B. P., Pei, R. S., & Xu, N. (2008). The antibacterial mechanism of carvacrol and thymol against *Escherichia coli*. *Letters in Applied Microbiology, 47*(3), 174–179.

Yin, H. B., Chen, C. H., Kollanoor-Johny, A., Darre, M. J., & Venkitanarayanan, K. (2015). Controlling *Aspergillus flavus* and *Aspergillus parasiticus* growth and aflatoxin production in poultry feed using carvacrol and trans-cinnamaldehyde. *Poultry Science, 94*(9), 2183–2190.

Yossa, N., Patel, J., Macarisin, D., Millner, P., Murphy, C., Bauchan, G., et al. (2014). Antibacterial activity of cinnamaldehyde and Sporan against *Escherichia coli* O157:H7 and *Salmonella*. *Journal of Food Processing and Preservation, 38*(3), 749–757.

Young, I., Rajić, A., Wilhelm, B. J., Waddell, L., Parker, S., & McEwen, S. A. (2009). Comparison of the prevalence of bacterial enteropathogens, potentially zoonotic bacteria and bacterial resistance to antimicrobials in organic and conventional poultry, swine and beef production: A systematic review and meta-analysis. *Epidemiology and Infection, 137*(9), 1217–1232.

Yusrizal, & Chen, T. C. (2003). Effect of adding chicory fructans in feed on broiler growth performance, serum cholesterol and intestinal length. *International Journal of Poultry Science, 2*, 214–219.

Chapter 3
Antibiotic Usage in Poultry Production and Antimicrobial-Resistant *Salmonella* in Poultry

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3.1 Introduction

We are living in an era where most of the antibiotics are now being increasingly reported for reduced efficacy. Antimicrobial resistance (AMR) is an emerging global health issue equally affecting developed as well as developing nations. AMR is expected to increase and cause considerable economic losses to the government exchequer across the globe. The total economic burden of AMR on the US economy is estimated to be approximately $20 billion in direct healthcare-related costs (Ventola 2015). A recent study estimated that medical cost attributable to antimicrobial-resistant infection (ARI) ranged from $18,588 to $29,069 per patient in a Chicago teaching hospital (Roberts et al. 2009). The Centers for Disease Control and Prevention (CDC) estimates that in the USA alone, at least two million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die each year as a direct result of these infections (CDC 2018). World Economic Forum's Global Risks 2014 report recently concluded that the potential impact and likelihood of AMR were as high as the threats posed by climate change and terrorism (World Economic Forum 2014). "Post-antibiotic era" is not an

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imaginary word anymore and seems to be a very real possibility with the number of bacterial pathogens being reported as resistant to almost all antibiotics on the shelf. The recent death of a female patient in the USA, suffering from carbapenem-resistant *Klebsiella pneumoniae*, confirms this concern (Branswell 2017). The patient was suffering from carbapenem-resistant *K. pneumoniae* and received treatment in India before being hospitalized in the USA. *K. pneumoniae* was found to be resistant to all available antimicrobial drugs including colistin (Branswell 2017; Dall 2017). This is one of the many cases of resistance to life-saving antibiotics identified in humans.

A range of factors are responsible for the emergence of AMR, some of which worth mentioning include overuse and inappropriate prescribing of antibiotics in human medicine, slow pace of discovery of newer antibiotics, use of antibiotics in food animals for growth promotion in unhygienic living conditions, poor healthcare infrastructure in low-income countries, and regulatory barriers (Ventola 2015; Landers et al. 2012). Of these, use of antibiotics in food animals for growth promotion and disease prevention has received considerable attention during the last few decades. Nevertheless, other factors listed above are equally important when considering contributions of all sectors in the emergence of AMR. Increasing demand for meat has resulted in establishment of more industrialized and intensive poultry operations (Sims 2008). These operations are usually overcrowded and lack adequate sanitary conditions (Hribar and Schultz 2010; Sims 2008). The sub-therapeutic use of antimicrobials in such farms has become a common management practice (Brooks 2011). Our ability to cure bacterial infections has decreased. Infections once easily treatable are now becoming fatal even with the high-end antibiotics (Ventola 2015). Medical doctors are now forced to use the reserve or last resort antibiotics to treat such infections. Once these antibiotics fail, we will not be left with many treatment options.

Low-dose administration of antimicrobials in poultry farms affects human, animal, and environmental health in several ways. Development of antibiotic-resistant pathogens is one such major risk to human and animal health. In particular, development of multidrug resistance (MDR, resistance to more than three classes of antimicrobials) in zoonotic pathogens like non-typhoidal *Salmonella* (NTS) and *Campylobacter* spp. is a serious concern (Skariyachan et al. 2016). *Salmonella* resistance to antimicrobial drugs is a critical issue of public concern and has gained wide scientific interest during the last few decades (Aarestrup 2015). This has resulted from a growing awareness that the wide use of antimicrobials in veterinary medicine and food animal production may compromise human health whether resistant bacteria develop in animals and are transferred to humans via the food chain or the environment. The prevalence of *Salmonella enterica* associated with poultry and poultry meat products has not been clearly documented, and this prevalence has an impact on public health and economic burdens (Rouger et al. 2017; Cosby et al. 2015). In this review, we discuss the use of antimicrobials in poultry production; different ways in which antibiotic usage can impact human, animal, and environmental health; as well as development and transmission of antimicrobial-resistant *Salmonella* in poultry production.

3.2 Antibiotic Use in Poultry Production

Global consumption of poultry meat is expected to overtake pork and beef in the next few years. One important reason is the increase in the global demand of inexpensive proteins, particularly among the developing nations due to rapidly rising population and per capita income. Chicken meat is fairly inexpensive compared to beef or pork. Therefore, in order to meet the rising demands of chicken meat, birds are reared in high-density farms with less space to roam. Sub-therapeutic administration of antimicrobials to prevent bacterial infections is a routine practice in such poultry farms to compensate overcrowding and unhygienic environments (Belanger 2015; Aguirre 2017). In European countries and the USA, restrictions exist regulating the use of antimicrobials in the animal feed for growth promotion; however, in several middle- and low-income countries, there are no such regulations (Van Boeckel et al. 2015). For example, many of the medically important antibiotics such as fluoroquinolones and cephalosporins are liberally used in Indian poultry farms, and antimicrobials are available over the counter without needing a medical or veterinary prescription (Bhushan et al. 2017).

A substantial increase of 35% in the global antibiotic consumption between 2000 and 2010 has been reported (Van Boeckel et al. 2014). Seventy six (76%) of the overall increase in the global consumption of antimicrobials between 2000 and 2010 was attributed to BRICS nations (Brazil, Russia, India, China, and South Africa). Among all nations, India ranked first in the consumption of antibiotics in 2010 with 12.9×10^9 units (10.7 units/person) followed by China (10.0×10^9 units and 7.5 units/person) and the USA (6.8×10^9 units and 22.0 units/person) (Van Boeckel et al. 2014). An increase in the global consumption of antimicrobials has resulted in the emergence of resistance in bacterial pathogens/strains that were previously considered susceptible (Kniel et al. 2018).

The substantial use of antimicrobials in poultry production is well-known in developed as well as developing nations. Use of antimicrobial agents is believed to result in more weight gain in a shorter duration of time yielding improved feed efficiency. A few decades earlier, a 1.13 kg broiler could be produced in 112 days; however, currently a 2.27 kg broiler can be produced in less than 50 days (The Pew Charitable Trusts 2013). Several theories exist about the role of antimicrobials in the increased and rapid weight gain in poultry. The most convincing explanation appears to be the increased energy efficacy of the gastrointestinal system following the consumption of the antibiotics (Cook 2004). A variety of microorganisms inhabit the poultry intestine and help in the breakdown of food. Although these microorganisms are useful in digestion and producing immune response, they also compete with the host for nutrients. Low-dose administration of antimicrobials in feed or water kills these gut microbes and minimizes the competition for nutrients between microorganisms and poultry (Cook 2004). This allows poultry to convert feed to muscle resulting in more rapid growth.

Poultry birds are often raised in high-density farms. These concentrated farms lead to easily shared commensal microflora as well as pathogenic microorganisms to each

other and in the environment (Levy and Marshall 2004). As a result, such large-scale farms require an aggressive pathogen prevention system in the form of sub-therapeutic administration of antimicrobials to poultry (Landers et al. 2012). In commercial poultry farms, antimicrobials are mainly used either as therapeutics drugs to treat sick flocks or growth promotion agents to speed up the growth (Singer and Hofacre 2006). Although some antimicrobials are exclusively used in animals, most of the antimicrobials used in animal farms belong to the same category as those used in human medicine (Landers et al. 2012). Moreover, a large portion of these constitutes "medically important" antimicrobials such as fluoroquinolones and cephalosporins, which are the drugs of choice in humans. Many of the last resort antibiotics such as colistin have been reported to be freely used for growth promotion in commercial poultry systems (Nhung et al. 2016). The situation appears to be worse in countries with no national regulations on use of antimicrobials in human and veterinary medicine.

Development of resistance in bacterial populations at poultry farms mainly occurs by two methods: (1) due to development point mutations or (2) by acquiring resistance from another bacterium (Walsh 2010). Genetic mutations in bacteria are spontaneous changes in the genetic makeup of the organism that render antibiotics ineffective, conveying a survival advantage to the mutated bacterial strains (Davies and Davies 2010). Mutations enable the bacteria to resist the effects of antimicrobials in several ways. One such mechanism is the production of enzymes that inactivate antibiotics. For example, many of the Gram-negative bacteria produce β-lactamases that inactivate the β-lactam antibiotics such as penicillins, cephalosporins, cephamycins, and carbapenems by hydrolyzing the β-lactam ring of these antibiotics and making the bacteria multidrug resistant (MDR). Bacteria can also acquire antimicrobial resistant genes from other bacteria by the process of "horizontal gene transfer (HGT)." Generally, antimicrobial resistance determinants are located on mobile genetic elements such as plasmids, integrons, and transposons, which possess the capability to transfer resistance traits to other bacteria (similar genus or different). Moreover, such bacteria can also exhibit multidrug resistance because of the presence of multiple resistance genes on a single mobile genetic element. For example, integrons are known to harbor a variety of antimicrobial resistant genes packed in a single gene cassette. These MDR bacteria originating in poultry farms eventually enter the human food chain by means of contaminated water, soil, and farm manure. Hence, it can be concluded that in modern intensive poultry farms, antimicrobials are mainly used to compensate the overcrowding, poor sanitary conditions, and absence of appropriate biosecurity measures.

3.3 Impact of Antibiotic Use on Poultry, Human, and Environmental Health

Imprudent use of antibiotics in poultry production affects human and environmental health in different ways. Firstly, low dose of antibiotics administered to poultry leads to the development of antimicrobial resistance in pathogenic bacteria by

exerting specific selection pressure over a long period of time. Secondly, occurrence of antibiotic residues in poultry meat poses a direct threat to public health. Another major issue is the transmission of antimicrobial resistance bacteria (ARB) and genes (ARGs) to the outer environment and eventually humans. Here, we will briefly discuss all these concerns. The most important consequence of using low-dose antibiotics in poultry is the development of AMR in bacterial pathogens. A major reason for development of AMR in poultry could be the practice of treating poultry in masses. Unlike human medicine, where infected persons are treated individually, poultry are treated collectively as a group. Antimicrobials are usually administered through drinking water in poultry farms, which results in unnecessary consumption of antibiotics by healthy birds. Ingestion of antibiotics by healthy birds over a long duration selects for development of AMR in bacterial pathogens such as *Salmonella* and *Campylobacter* while having a positive effect on the health of the poultry at the same time (Singer and Hofacre 2006). Susceptible microorganisms are killed or inhibited, and the organisms which could resist the presence of an antimicrobial become resistant and proliferate. Several studies have compared the prevalence of AMR pathogens in conventional and antibiotic free or organic farms (Keelara et al. 2013; Zhu et al. 2013). It was assumed that the differences in the prevalence of antimicrobial resistance bacteria are mainly due to the different antimicrobial usage practices in such farms (Thanner et al. 2016; Berendsen et al. 2015; Keelara et al. 2013; Zhu et al. 2013). However, some studies have reported the presence of AMR bacteria in antibiotic-free farms (Keelara et al. 2013; Young et al. 2009).

Approximately 30–90% antibiotics consumed by the food animals are excreted in urine and manure (Berendsen et al. 2015). Manure generated in livestock farms serves as an efficient reservoir of antimicrobial-resistant bacteria and genes. In animal farms, manure is usually spread on land used for agricultural purposes. Spreading manure in soils facilitates the transfer of antimicrobial resistance bacteria (ARB) and antimicrobial resistant genes (ARGs) by horizontal gene transfer (HGT) to the soil microbiome (Thanner et al. 2016; Von Wintersdorff et al. 2016). The manure-enriched soil can further contaminate the water supply, fresh produce, and food animals eventually creating a public health burden (Marti et al. 2013). Recently, Kumar et al. (2018) demonstrated the persistence of multidrug methicillin-resistant *Staphylococcus sciuri* (MDR-MRSS) in the environment after land application of manure in commercial swine farms. Prevalence of MDR-MRSS was found to be highest in the soil samples collected after 2 h of manure application on Day 0, which subsequently decreased on samplings done on 7, 14, and 21 days. Approximately, 95.5% of the *S. sciuri* isolates were multidrug resistant (MDR) (Kumar et al. 2018). A study recently detected 149 unique resistance genes at three large-scale (10,000 animals per year) commercial swine farms in China. The 63 most prevalent ARGs were enriched 192-fold up to 28,000-fold compared to the antibiotic-free manure or soil controls (Zhu et al. 2013). Several studies have identified poultry litter as a source of antibiotic resistance bacteria and genes (Hruby et al. 2018; Cook et al. 2014; Dhanarani et al. 2009; Diarrassouba et al. 2007; Nandi et al. 2004). Poultry litter is a valuable nutrient source for crop production but can also be a route of environmental contamination when laden with AMR bacteria. Isolation of foodborne pathogens such as *Staphylococcus*, *E. coli*, and *Salmonella* has been reported

from poultry litter and other samples in poultry farms. Recently, Brower et al. (2017) reported that broiler farms were associated with a higher prevalence of resistance, including ESBL-producing *Enterobacteriaceae* and multidrug resistance, than layer farms in India. Authors surveyed 18 randomly selected poultry farms (nine layers and nine broilers) for the presence of *E. coli* and found that broiler farms were 2.2–23 times more likely to be resistant to *E. coli* strains compared to the layer farms. They also reported increased prevalence of multidrug-resistant *E. coli* (94%) in broiler farms than layer farms (60%). Nandi et al. (2004) reported that Gram-positive bacteria were the major reservoir of class 1 antibiotic resistance integrons in poultry litter and constitute approximately greater than 85% of the litter community compared with *Enterobacteriaceae* that comprise less than 2% of this ecosystem.

Linking antimicrobial use in food animals in general, and poultry in particular, resulting in AMR infections in humans is complex. Most of the studies seeking such link explore the presence of AMR bacteria on retail chicken meat samples. The general assumption in such studies is that the bacteria found on chicken meat originate from the poultry farm and have been picked up by birds in the farm settings (Singer and Hofacre 2006). Also, the level of resistance to antimicrobials reflects the antimicrobial management practices in poultry farms. Providing pinpoint evidence linking an AMR infection in humans to the pathogens originating in poultry farms is unrealistic and unnecessary; the potential hazards of overuse of antimicrobials are well-known. Moreover, an increase in the prevalence of AMR in pathogens following long-term antimicrobial usage in farms has been reported. For example, in Canada, following the voluntary 2005 withdrawal of cephalosporins in poultry industry resulted in decreased ceftiofur resistance in animals and humans. Before such ban, in 2003, high rates of cephalosporin resistance in *Salmonella* Heidelberg isolated from poultry, retail chicken meat, and humans were observed in Quebec, Canada (Dutil et al. 2010). Treatment with enrofloxacin or sarafloxacin in poultry has been reported to result in increased resistance in *C. jejuni* (McDermott et al. 2002). These authors experimentally infected poultry with susceptible *C. jejuni* strains and treated the birds with enrofloxacin or sarafloxacin. They observed an increase in the ciprofloxacin MICs from 0.25 μg/ml to 32 μg/ml within the 5-day treatment time frame. These results suggest that use of fluoroquinolones in poultry rapidly selects for resistant *Campylobacter* and, if transmitted to humans, may result in treatment failure with fluoroquinolones. Another convincing evidence is the case of Australia, where fluoroquinolones have never been licensed for use in food animals. In a study, only 12 out of 370 *Campylobacter* isolated from human infections were found fluoroquinolone resistant. Ten of these cases were patients who had recently travelled outside Australia (Unicomb et al. 2003). It was suggested that the absence of human and locally acquired *Campylobacter* infections linked to fluoroquinolone-resistant organisms was most likely due to the ban on use of fluoroquinolones in Australian poultry industry. Fluoroquinolone resistance in humans acquired from meats has not been reported from Australia.

Humphrey et al. (2005) compared the prevalence of ciprofloxacin-resistant *Campylobacter* spp. in commercial poultry flocks before fluoroquinolone treatment (1–5 days before the start of treatment), during treatment (2–5 days after the start of treatment), and after treatment (weekly for up to 4 weeks posttreatment) until the flock

was slaughtered. Authors reported that large numbers of ciprofloxacin-resistant *Campylobacter* emerge rapidly in commercial broiler chickens after treatment with a fluoroquinolone and a proportion of such strains persisted for up to 4 weeks after treatment was discontinued. The percentage of ciprofloxacin-resistant isolates during treatment was significantly ($P < 0.001$) higher than that before treatment. However, they also found a small proportion of ciprofloxacin-resistant strains prior to fluoroquinolone exposure and suggested that such resistant strains are established in the farm environment and that flocks may be exposed to them even in the absence of antibiotic exposure. Detection of ciprofloxacin-resistant strains prior to fluoroquinolone exposure highlights the role that environmental reservoirs play in the dissemination of antimicrobial resistance. Papadopoulos et al. (2015) reported a multidrug-resistant clone of *Salmonella enterica* serovar Hadar circulating among humans, poultry, and foods of animal origin in Greece during 2007–2010 which represented 47% of all *S.* Hadar isolates identified during the study period. They suggested that this particular clone (PFGE profile SHADXB.0001) was an endemic clone as it was frequently isolated from hatcheries, breeder flocks, laying hens, broilers, foods of animal origin, and humans and was found during each year of the study period. Smith et al. (1999) reported that similar *C. jejuni* strains were isolated from chicken products and domestically acquired infections in Minnesota residents.

Another method of providing such evidence is to screen the people living close to the livestock operations for antimicrobial-resistant pathogens or to compare conventional and antibiotic-free livestock operations. People working and living close to animal feeding operations are particularly at a greater risk of acquiring AMR infections. Recently, a study evaluated differences in occupational risk of methicillin-resistant *Staphylococcus aureus* (MRSA) to the farm workers working in industrial livestock operations (ILO) and antibiotic-free livestock operations (AFLO) (Rinsky et al. 2013). Although the prevalence of *Staphylococcus aureus* and MRSA was similar among workers of both ILO and AFLO farms, the *S. aureus* clonal complex (CC) 398, which is a livestock-associated MRSA clone, was predominately detected among ILO workers compared to AFLO workers. Moreover, only ILO workers carried scn-negative MRSA CC398 and scn-negative MDRSA CC398 strains, which confirms the presence of LA-MRSA in ILO workers. In a study from the USA, Casey et al. (2013) reported higher risk of community-associated MRSA infection, skin and soft-tissue infection in the human populations living close to the high-density swine production systems. These studies, although done in and around swine farms, strengthen the hypothesis that antibiotic usage in food animal farms puts humans working and living close to such farms at a severe risk of acquiring AMR infections.

3.4 Recent Case of Transferrable Colistin Resistance and Poultry

Colistin is a last resort antibiotic preserved to treat critical human cases of carbapenem-resistant *Enterobacteriaceae* such as *Escherichia coli*, *Klebsiella*, and *Acinetobacter*. In the past, colistin was not used in human medicine fearing the

renal side effects and low renal clearance; however, it is now being used to treat extremely resistant cases of Gram-negative bacteria (Wang et al. 2018; Boucher et al. 2009). Until 2015, resistance to colistin was linked only to chromosomal mutations. However, a recent discovery of a plasmid-mediated transferrable form of colistin resistance in *E. coli* isolated from food animals, retail meats, and human clinical cases in China has opened doors to a new threat (Liu et al. 2016). Liu et al. (2016) reported detection of an *mcr*-1 gene coding colistin resistance in *E. coli* isolated from 15% raw pork and chicken meat, 21% food animals, and 1% hospital patients. The presence of colistin resistance on mobile genetic elements is a significant public health risk, as resistance genes encoding colistin resistance can easily spread to other domains by horizontal gene transfer (HGT) (Wang et al. 2018). Moreover, simultaneous detection of *mcr*-1 gene from food animals, retail meats, and human clinical cases suggest interplay of different host environments and further complicate the situation (Doumith et al. 2016). Since its first reporting, *mcr*-1 gene and its variants (*mcr*-2, *mcr*-3, *mcr*-4, *mcr*-5, *mcr*-6, *mcr*-7.1) have been reported from several countries spanning over five continents (Yang et al. 2018; Chen et al. 2018; Hembach et al. 2017; Ovejero et al. 2017; Guenther et al. 2017; Fernandes et al. 2017; Rossi et al. 2017; Zurfuh et al. 2016).

Unregulated use of colistin in animal farms in China is thought to be the precursor of emergence of colistin resistance in bacterial isolates of food animal origin. China is one of the highest users of colistin in agriculture (Liu et al. 2016). The global demand for colistin is expected to touch 16,500 tons by the year 2021 at an average annual growth rate of 4.75%, and much of this anticipated increase is attributed to increased use of colistin in agriculture in China. China is estimated to be utilizing 8000 tons of 12,000 tons global production of colistin per year. The European Union and North America imported 480 tons and 700 tons of colistin, respectively, from China in 2015 (Liu et al. 2016). Following the emergence of the *mcr*-1 gene, Chinese government banned the use of colistin as a growth promoter in 2016 to reduce colistin usage in food animal farms (Founou et al. 2016).

However, there is no respite in the consumption and sale of colistin in low- and middle-income countries. Recently, 2800 tons of colistin was tracked being shipped from China to countries such as India, Nepal, Malaysia, Thailand, Vietnam, South Korea, Guatemala, Colombia, and Mexico for use in poultry farms (Davies and Walsh 2018; Nhung et al. 2016). This total could be higher as the products are sold with a brand name instead of being labelled as colistin. The situation could be far more threatening in countries such as India and some countries in Southeast Asia, which are considered as the epicenter of antimicrobial resistance. India's consumption of antibiotics in chickens is predicted to rise five-fold by 2030 compared to 2010, while globally the amount used in animals is expected to rise by 53% (Davies and Meesaraganda 2018). Over-the-counter availability of antibiotics, huge human population, increasing demand for low cost protein, open defecation in some rural areas, huge pharmaceutical industry, and most importantly no strict laws on antibiotic usage and sale are the main issues which may result in rapid emergence of colistin resistance in India (Davies and Meesaraganda 2018). Although the Indian government recently introduced a national action plan to curb the antibiotic misuse in animal and human medicine, it is not backed by any regulatory action (Dutta

2017). If not banned as a growth promoter in poultry, colistin resistance will likely spread on the poultry farms and will enter the human population through contaminated chicken meat, infected farm workers, and bacteria-laden manure and flies. The identical situation occurs in several countries over the world.

3.5 Antimicrobial-Resistant *Salmonella* in Poultry

One of the significant bacterial infection problems in poultry production is salmonellosis. Moreover, *Salmonella* resistance to antimicrobial drugs has double generally a critical issue of public concern and become a scientific interest during the last few decades (Aarestrup 2015). This resulted from a growing nervousness that the wide use of antimicrobials in veterinary medicine and food animal production may compromise human health whether resistant bacteria are to develop in animals and are to be transferred to humans via the food chain or the environment. The prevalence of *Salmonella enterica* associated with poultry and poultry meat products has not been clearly documented, and this prevalence has an impact on public health and economic burdens (Rouger et al. 2017; Cosby et al. 2015). Non-typhoidal *Salmonella* (NTS) is the leading cause of bacterial foodborne illness in the USA which is estimated 1.2 million illnesses, 19,000 hospitalizations, and 380 deaths, every year (CDC 2014). *Salmonella* causes an approximate economical loss of over 14 billion dollars a year (Cosby et al. 2015). In 2014, it was estimated that 360,000 (30%) of foodborne illnesses are attributed to meat and poultry products which is a 9.3% decrease when compared to 2010 (IFSAC 2015; FSIS 2014). Almost 41,930 cases of non-typhoidal foodborne salmonellosis are confirmed annually with an estimated total number of 1 million cases of foodborne salmonellosis not reported. Although human salmonellosis is generally a self-limiting disease and treatment would be needed only in severe cases, the increase of MDR *Salmonella* including fluoroquinolones and extended-spectrum β-lactams (ESBL) makes infections more complicated (Fischer et al. 2012). Additionally, MDR *Salmonella* infections lead to treatment failure, prolonged hospitalization, and an increase of economic loss in public health. The emergence of antimicrobial-resistant *Salmonella* recovered from meat products has heightened concerns regarding antimicrobial use in food animal production.

In the USA, more than 80% of *Salmonella* isolated from meat products including chicken, pork, and beef showed resistance to at least one antimicrobial, and approximately 50% showed resistance to at least three antimicrobials (Hur et al. 2012; White et al. 2002). High prevalence of resistant *Salmonella* to penicillin, oxacillin, clindamycin, vancomycin, erythromycin, and ampicillin was evident in more than 80% in chicken carcass in Turkey (Yildirim et al. 2011). *Salmonella* isolated from pork, duck, and chicken in Sichuan, China, demonstrated high antimicrobial resistance rates observed for tetracycline, sulfamethoxazole/trimethoprim, nalidixic acid, and spectinomycin, and class 1 integrons were also detected (Li et al. 2013). Another research in China reported that 45% of *Salmonella* isolates from retail raw poultry sampling were resistant to 1–5 antimicrobials, 29% were resistant to 6–10 agents, and 22% were resistant to 11–15 agents; only 4% of the isolates were susceptible (Yang et al. 2014). In Southeast Asia, *S.*

enterica is also commonly found in chickens, pigs, cows, farm workers, and diarrheal children (Van et al. 2012; Padungtod and Kaneene 2006). Sinwat et al. (2015) reported that *Salmonella* isolated from raw pork, raw chicken, and humans exhibited high resistance to sulfamethoxazole (96.4%) and streptomycin (93.2%). A report from Southern Thailand stated that *Salmonella* isolates from pork and vegetables were most resistant to tetracycline with 77 and 33%, respectively, while the *Salmonella* isolates from chicken meat were most resistant to streptomycin (92%). In addition, 68% of pork isolates and 84% of chicken meat isolates were MDR strains (Lertworapreecha et al. 2012). In Vietnam, 78% of *Salmonella* isolates from retail meat samples were resistant to at least one class of antimicrobials, and the highest frequency of resistance was detected against tetracycline, sulfonamides, streptomycin, ampicillin, and chloramphenicol (Thai et al. 2012; Thai and Yamaguchi 2012). Moreover, chicken isolates exhibited higher resistance to antimicrobials than pork isolates (Thai et al. 2012). The surveillance in commercial poultry farms in the USA conducted by the National Antimicrobial Resistance Monitoring System (NARMS) reported that 63% of *Salmonella* were pan-susceptible. However, *Salmonella* isolates with resistance to only streptomycin and together with other antimicrobials were the most prevalent (36.3%) antimicrobial resistance phenotype observed (Liljebjelke et al. 2017).

3.6 Spread of Antimicrobial-Resistant *Salmonella* to Food Communities

Salmonella is the leading bacterial cause of foodborne illness, and the dissemination of antimicrobial-resistant *Salmonella* through the food chain has critical impacts on treatment failure in human salmonellosis. Exposure to poultry meat has been linked to *Salmonella* illness. Due to the risk of human infection mostly associated with poultry products and the prevalence of antimicrobial resistance, *Salmonella* also poses a significant challenge to commercial poultry production and the national health goal (Liljebjelke et al. 2017). Food Safety and Inspection Service (FSIS) reveals that *Salmonella* serotype identification generated through the Pathogen Reduction and Hazard Analysis and Critical Control Point (PR/HACCP) program helps to monitor trends of isolates identified in several products as a proactive guide to make better decisions affecting food safety and public health (FSIS 2017).

3.6.1 Antimicrobial Resistance of Poultry-Associated Salmonella *Serovars*

Serotyping is a process by which the types of *Salmonella* can be differentiated based on their surface antigens: O and H antigens following the Kaufmann-White scheme maintained by the World Health Organization (WHO) (Guibourdenche et al. 2010; Grimont and Weill 2007). The USDA-FSIS uses the Centers for Disease Control

and Prevention (CDC) criteria on serotype identification from human salmonellosis and compares the data to *Salmonella* serovars isolated from meat and poultry products. Some *Salmonella* serovars are not common in human patients but usually found in meat and poultry products (FSIS 2014, 2017). However, some of the serovars are frequently responsible for human clinical cases of salmonellosis and occur generally in meat, poultry products, and non-food sources (FSIS 2014, 2017).

During the period 2002–2012, the 12 most prevalent poultry-associated *Salmonella* serovars were frequently and consistently isolated from poultry products in the USA including Kentucky; Enteritidis; Heidelberg; Typhimurium; 4,[5],12:i:-; Montevideo; Infantis; Schwarzengrund; Hadar; Mbandaka; Thompson; and Senftenberg (FSIS 2017; Shah et al. 2017). This data was in accordance to the previous report from Jackson et al. (2013). They clarified that serovars Enteritidis, Heidelberg, and Hadar which were attributed to eggs and poultry were predominantly responsible for *Salmonella* outbreaks in the USA during 1988–2008 (Jackson et al. 2013). Moreover, serovars Enteritidis, Montevideo, Typhimurium, Infantis, and Heidelberg recovered in meat and poultry products were also among the predominant serovars reported as the cause of human salmonellosis by the CDC (Shah et al. 2017).

Several serovars identified in poultry are more likely reported as MDR including Heidelberg, Typhimurium, Kentucky, and Senftenberg, whereas Enteritidis, Montevideo, Schwarzengrund, Hadar, Infantis, Thompson, and Mbandaka are generally found pan-susceptible or show resistance to fewer antimicrobials. There also appears to be an international spread of a few MDR serovars including Kentucky, Schwarzengrund, Hadar, Thomson, Senftenberg, and Enteritidis, which may pose significant challenges to the public health (Shah et al. 2017). There were within and between farm differences in the antimicrobial susceptibilities of *Salmonella*, and some of these differences were linked to specific serovars (Liljebjelke et al. 2017). However, farm differences were not linked to antimicrobial usage. Preventing vertical transmission of antimicrobial-resistant *Salmonella* would reduce carcass contamination with antimicrobial-resistant *Salmonella* and subsequently human risk exposure (Liljebjelke et al. 2017).

3.6.2 The Safety in Food Commodities

According to the recent surveillance from CDC National Outbreak Reporting System from 2009 to 2014, the most frequent food sources contributed to *Salmonella* infection were animal-origin food (64%) with chicken as the first rank (14%) followed by eggs (11%) (Dewey-Mattia et al. 2016). Among those chicken, young chicken carcasses are the product with the greatest potential to cause exposure of the public to *Salmonella* (FSIS 2015). This is the reason why the FSIS and CDC continue to direct its resources toward implementing and revising performance standards to reduce the load of *Salmonella* in chicken carcasses. Since 1998, *Salmonella* Kentucky has been considered the most common serotype identified among young

chicken isolates. However, *S.* Kentucky from chicken carcasses is not among the serotypes commonly associated with human salmonellosis in the USA (FSIS 2017; Shah et al. 2017). *S.* Enteritidis was the second most common serovar identified in young chicken carcasses and is recently the most common serovar causing human illness (CDC 2013; Foley et al. 2011). Eggs are also reported to be the most frequent food commodity associated with *S.* Enteritidis outbreaks (Jackson et al. 2013). However, since 2010, there has been a steady decline in the prevalence of *S.* Enteritidis in young chicken carcasses (14.8–9.4%) (CDC 2013).

From farm to table, there are numerous possible routes of *Salmonella* dissemination and contamination in poultry. During the time that chicks are hatched through growing stage, transportation, processing, storage, preparation, and finally consumption, the product could be contaminated through exposure to different materials and sources (Founou et al. 2016). Examination of each step is necessary as well as an examination of the overall process to create effective mitigation countermeasures against contamination and prevent infection.

3.7 Transmission of Antimicrobial-Resistant *Salmonella* in Poultry

In addition to the birds, the poultry environment can also become a source of AMR transmission. Resistant *Salmonella* as well as other foodborne bacteria can move out of poultry farms into the environment through waste disposal (Bhushan et al. 2017). Poultry litter or manure has been implicated as a source of resistant *Salmonella* bearing linkages to the transmission of AMR into the outside environment. Several studies have reported the isolation of AMR *Salmonella* from the environmental samples including chicken feces, poultry litter, boot swabs, and nearby surface water and groundwater which significantly are more resistant to antimicrobials than isolates from other sources (Mattiello et al. 2015; Gao et al. 2014; Furtula et al. 2013).

The clonal spread and horizontal gene transfer (HGT) are considered as major routes of AMR distribution among *Salmonella* (Warnes et al. 2012; Helmuth 2000). Studies from several parts of the world reported the distribution of multiple plasmid-located AMR genes by HGT including plasmids harboring extended-spectrum β-lactamase (ESBL) genes (*bla*CTX, *bla*SHV, *bla*CMY, and *bla*TEM) or *amp*C and plasmid-mediated quinolone resistance (PMQR) genes (*qnr*A, *qnr*B, and *qnr*S) in *Salmonella* among animal, human, and environmental sources (Pornsukarom and Thakur 2017; Chen et al. 2016; McCollister et al. 2016; Accogli et al. 2013; Dolejska et al. 2013). Another recent example of plasmid-mediated transfer is mobile colistin resistance gene (*mcr*-1) (Doumith et al. 2016). There is a broad host range dissemination of *mcr*-1-containing plasmids maintained in the *Enterobacteriaceae* family in human, animal, and food products and recently being reported from different countries over the world (Doumith et al. 2016; Gao et al. 2016; Liu et al. 2016). The latest

global health expansion in plasmids conferred resistance in carbapenem-resistant *Enterobacteriaceae* (CRE) which recovered from the environment of a livestock production area in the USA (Mollenkopf et al. 2017; Gao et al. 2016; Liu et al. 2016).

3.8 Antimicrobial Resistance Mechanisms of *Salmonella*

Salmonella becomes antimicrobial resistant due to various mechanisms including enzymatic antimicrobial inactivation, modification/protection of target sites, limiting antimicrobial access to microbial cell, and active efflux (Walsh 2010; Alekshun and Levy 2007). Among these mechanisms, the presence of integrons and overexpression of active efflux are known as the main causes of MDR in *Salmonella* (Blair et al. 2015; Lee et al. 2002). Integrons are mobile genetic elements located on *Salmonella* chromosome or conjugative plasmid. In Gram-negative bacteria, a class 1 integron is the most common type carrying various resistance gene cassettes and responsible for horizontal transfer (Wellington et al. 2013; Fluit and Schmitz 2004; Lee et al. 2002).

Recently, new mechanisms of resistance have resulted in the simultaneous development of resistance to several antimicrobial classes creating critical MDR *Salmonella* strains, also known as "superbugs" such as the MDR *Salmonella* Typhimurium phage type DT104, which is disseminated worldwide and carries the *Salmonella* genomic island 1 (SGI1) encoding various resistance antimicrobials (Ferri et al. 2017). The presence of SGI1, which is chromosomally located, typically limits to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline resistances. Because of SGI1, *Salmonella* is able to maintain the resistance determinants without selective pressure. Thus, it is considered as a risk for rapid dissemination of AMR (Walsh 2010; Mulvey et al. 2006). Class I and class II integrons have been recovered in *Salmonella*. Class I integrons are primarily in the *Salmonella* genomic islands, while class II integrons are embedded in the TN7 transposon family but have not been clearly described (Fluit 2005). Liljebjelke et al. (2017) reported that resistance to streptomycin and sulfadimethoxine appeared to be linked to the transposon TN21. Unlike integrons, active efflux pumps are not structurally related; thus the cross-resistance to MDR can occur. The efflux pumps decrease the gradients of antimicrobial to insufficient level which is not harmful to bacterial cells and supports the mechanism of MDR (Walsh 2010). The AMR genes occur via mutation in DNA and horizontal gene transfers by transformation, transduction, and conjugation (Fluit and Schmitz 2004; Helmuth 2000).

3.9 Conclusions

There are several issues responsible for increased prevalence of MDR pathogens such as indiscriminate use of antimicrobials in food animals for growth promotion and diseases prevention, over-the-counter availability of antimicrobials in some

countries, and incorrect prescription practices. Among these, the overuse of antimicrobials in the food animals, particularly poultry, receives the spotlight. Increased demand for poultry meat has resulted in poultry being raised in large-scale high-density intensive farms where large numbers of birds are kept together. These high-density farms result in easy sharing of commensal microbiota and pathogenic microorganisms in the environment and necessitate sub-therapeutic administration of antimicrobials. We suggest eliminating or significantly reducing the use of non-therapeutic and sub-therapeutic administration of antimicrobials in poultry production. Such a restriction might result in increased prices of chicken meat, but eventually poultry producers will be forced to improve overall hygiene and biosecurity measures at poultry farms. There is also a need for constant veterinary surveillance of AMR and use of antimicrobials in poultry farms; raising awareness among professionals, farm workers, livestock handlers, and lab technicians; and designing policies on the national drug regulatory authorities in the animal health sector.

To mitigate the development of resistance, some countries have restricted antimicrobial use in feed, and some countries advocate measures of antimicrobial use in human health as well as livestock production. However, monitoring of the quantities of antimicrobials used in animal production is limited to only a few countries. Most countries have administrative procedures for marketing authorization, but the extent to which it is applied varies markedly between countries. Awareness within countries on the possible adverse effects of the use of antimicrobial drugs in animal husbandry varies from good to negligible. Alternative means to control the increase of antimicrobial resistance are growth-promoting and prophylactic uses instead of antimicrobials in agriculture including improved management practices, wider use of vaccines, and introduction of probiotics. Monitoring programs, prudent use guidelines, and educational campaigns also provide approaches to minimize the further development of antimicrobial resistance. While there is still no consensus on the degree to which usage of antimicrobials in animals contributes to the development and dissemination of antimicrobial resistance in human *Salmonella*, experiential evidence and epidemiological and molecular studies point to a relationship between antimicrobial use and the emergence of resistant bacterial strains in animals, and their spread to humans, via the food chain especially poultry production.

References

Aarestrup, F. M. (2015). The livestock reservoir for antimicrobial resistance: A personal view on changing patterns of risks, effects of interventions and the way forward. *Philosophical Transactions of the Royal Society B: Biological Sciences, 370*(1670), 20140085.

Accogli, M., Fortini, D., Giufrè, M., Graziani, C., Dolejska, M., Carattoli, A., et al. (2013). IncI1 plasmids associated with the spread of CMY-2, CTX-M-1 and SHV-12 in *Escherichia coli* of animal and human origin. *Clinical Microbiology and Infection, 19*(5), E238.

Aguirre, E. (2017). Contagion without relief: Democratic experimentalism and regulating the use of antibiotics in food-producing animals. *UCLA Law Review, 64*, 550.

Alekshun, M. N., & Levy, S. B. (2007). Molecular mechanisms of antibacterial multidrug resistance. *Cell, 128*(6), 1037–1050.

Belanger, A. (2015). A holistic solution for antibiotic resistance: Phasing out factory farms in order to protect human health. *Journal of Health & Biomedical Law, 11*, 145.

Berendsen, B. J., Wegh, R. S., Memelink, J., Zuidema, T., & Stolker, L. A. (2015). The analysis of animal faeces as a tool to monitor antibiotic usage. *Talanta, 132*, 258–268.

Bhushan, C., Khurana, A., Sinha, R., & Nagaraju, M. (2017). *Antibiotic resistance in poultry environment: Spread of resistance from poultry farm to agricultural field*. New Delhi, India: Centre for Science and Environment.

Blair, J. M., Webber, M. A., Baylay, A. J., Ogbolu, D. O., & Piddock, L. J. (2015). Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology, 13*(1), 42–49.

Boucher, H. W., Talbot, G. H., Bradley, J. S., Edwards, J. E., Gilbert, D., Rice, L. B., et al. (2009). Bad bugs, no drugs: No ESKAPE! An update from the Infectious Diseases Society of America. *Clinical Infectious Diseases, 48*(1), 1–12.

Branswell, H. (2017). *Woman killed by a superbug resistant to every available antibiotic*. Retrieved March 2018, from https://www.scientificamerican.com/article/woman-killed-by-a-superbug-resistant-to-every-available-antibiotic/

Brooks C. (2011). *Meat's environmental impact*. Stanford Woods: Institute for the Environment. Retrieved March 2018, from https://woods.stanford.edu/news-events/news/meats-environmental-impact

Brower, C. H., Mandal, S., Hayer, S., Sran, M., Zehra, A., Patel, S. J., et al. (2017). The prevalence of extended-spectrum beta-lactamase-producing multidrug-resistant *Escherichia coli* in poultry chickens and variation according to farming practices in Punjab, India. *Environmental Health Perspectives, 125*(7), 238–241.

Casey, J. A., Curriero, F. C., Cosgrove, S. E., Nachman, K. E., & Schwartz, B. S. (2013). High-density livestock operations, crop field application of manure, and risk of community-associated methicillin-resistant *Staphylococcus aureus* infection in Pennsylvania. *JAMA Internal Medicine, 173*(21), 1980–1990.

Centers for Disease Control and Prevention (CDC). (2013). *Vital signs: Incidence and trends of infection with pathogens transmitted commonly through food – foodborne diseases active surveillance network, 10 U.S. Sites, 1996–2012*. Retrieved February 2018, from http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6215a2.htm?s_cid=mm6215a2_w

Centers for Disease Control and Prevention (CDC). (2014). *Foodborne disease active surveillance network*. Retrieved January 2018, from http://www.cdc.gov/foodnet/trends/2014/number-of-salmonella-infections-by-serotype-2014.html

Centers for Disease Control and Prevention (CDC). (2018). *Antibiotic resistance threats in the United States*. Retrieved February 2018, from http://www.cdc.gov/drugresistance/threat-report-2013

Chen, W., Fang, T., Zhou, X., Zhang, D., Shi, X., & Shi, C. (2016). IncHI2 plasmids are predominant in antibiotic-resistant *Salmonella* isolates. *Frontiers in Microbiology, 7*, 1566.

Chen, L., Zhang, J., Wang, J., Butaye, P., Kelly, P., Li, M., et al. (2018). Newly identified colistin resistance genes, *mcr*-4 and *mcr*-5, from upper and lower alimentary tract of pigs and poultry in China. *PLoS One, 13*(3), e0193957.

Cook, M. E. (2004). Antibodies: Alternatives to antibiotics in improving growth and feed efficiency. *Journal of Applied Poultry Research, 13*(1), 106–119.

Cook, K. L., Netthisinghe, A. M. P., & Gilfillen, R. A. (2014). Detection of pathogens, indicators, and antibiotic resistance genes after land application of poultry litter. *Journal of Environmental Quality, 43*(5), 1546–1558.

Cosby, D. E., Cox, N. A., Harrison, M. A., Wilson, J. L., Buhr, R. J., & Fedorka-Cray, P. J. (2015). *Salmonella* and antimicrobial resistance in broilers: A review. *Journal of Applied Poultry Research, 24*(3), 408–426.

Dall, C. (2017). *Pan-resistant CRE reported in Nevada*. Retrieved March 2018, from http://www.cidrap.umn.edu/news-perspective/2017/01/pan-resistant-cre-reported-nevada

Davies, J., & Davies, D. (2010). Origins and evolution of antibiotic resistance. *Microbiology and Molecular Biology Reviews, 74*(3), 417–433.

Davies, M., & Meesaraganda, R. (2018). *A game of chicken: How India's poultry farms are spawning global superbugs*. Retrieved April 2018, from https://www.thebureauinvestigates.com/stories/2018-01-30/a-game-of-chicken-how-indian-poultry-farming-is-creating-global-superbugs

Davies, M., & Walsh, T. R. (2018). A colistin crisis in India. *Lancet Infectious Diseases, 18*(3), 256–257.

Dewey-Mattia, D., Manikonda, K., & Vieira, A. (2016). *Surveillance for foodborne disease outbreaks–United States, 2014: Annual report*. Retrieved March 2018, from https://stacks.cdc.gov/view/cdc/40019

Dhanarani, T. S., Shankar, C., Park, J., Dexilin, M., Kumar, R. R., & Thamaraiselvi, K. (2009). Study on acquisition of bacterial antibiotic resistance determinants in poultry litter. *Poultry Science, 88*(7), 1381–1387.

Diarrassouba, F., Diarra, M. S., Bach, S., Delaquis, P., Pritchard, J., Topp, E., et al. (2007). Antibiotic resistance and virulence genes in commensal *Escherichia coli* and *Salmonella* isolates from commercial broiler chicken farms. *Journal of Food Protection, 70*(6), 1316–1327.

Dolejska, M., Villa, L., Hasman, H., Hansen, L., & Carattoli, A. (2013). Characterization of IncN plasmids carrying *bla* CTX-M-1 and *qnr* genes in *Escherichia coli* and *Salmonella* from animals, the environment and humans. *Journal of Antimicrobial Chemotherapy, 68*(2), 333–339.

Doumith, M., Godbole, G., Ashton, P., Larkin, L., Dallman, T., Day, M., et al. (2016). Detection of the plasmid-mediated *mcr*-1 gene conferring colistin resistance in human and food isolates of *Salmonella enterica* and *Escherichia coli* in England and Wales. *Journal of Antimicrobial Chemotherapy, 71*(8), 2300–2305.

Dutil, L., Irwin, R., Finley, R., Ng, L. K., Avery, B., Boerlin, P., et al. (2010). Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. *Emerging Infectious Diseases, 16*(1), 48–54.

Dutta, S. S. (2017). India launches strategy to curb antimicrobial resistance. *BMJ, 357*, j2049.

Fernandes, M. R., Sellera, F. P., Esposito, F., Sabino, C. P., Cerdeira, L., & Lincopan, N. (2017). Colistin-resistant *mcr*-1-positive *Escherichia coli* on public beaches, an infectious threat emerging in recreational waters. *Antimicrobial Agents and Chemotherapy, 61*(7), e00234–e00217.

Ferri, M., Ranucci, E., Romagnoli, P., & Giaccone, V. (2017). Antimicrobial resistance: A global emerging threat to public health systems. *Critical Reviews in Food Science and Nutrition, 57*(13), 2857–2876.

Fischer, J., Rodríguez, I., Schmoger, S., Friese, A., Roesler, U., Helmuth, R., et al. (2012). *Salmonella enterica* subsp. *enterica* producing VIM-1 carbapenemase isolated from livestock farms. *Journal of Antimicrobial Chemotherapy, 68*(2), 478–480.

Fluit, A. C. (2005). Towards more virulent and antibiotic-resistant *Salmonella*? *FEMS Immunology and Medical Microbiology, 43*(1), 1–11.

Fluit, A. C., & Schmitz, F. J. (2004). Resistance integrons and super-integrons. *Clinical Microbiology and Infection, 10*(4), 272–288.

Foley, S. L., Nayak, R., Hanning, I. B., Johnson, T. J., Han, J., & Ricke, S. C. (2011). Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. *Applied and Environmental Microbiology, 77*(13), 4273–4279.

Food Safety and Inspection Service (FSIS), United States Department of Agriculture (USDA). (2014). *A year in review – 2014*. Retrieved January 2018, from http://www.fsis.usda.gov/wps/wcm/connect/6f85bdf5-475a-4c15-8060-7d478ed1fd99/FY-2014-Year-in-Review.pdf?MOD=AJPERES

Food Safety and Inspection Service (FSIS), United States Department of Agriculture (USDA). (2015). *The FSIS Salmonella action plan: A year one update*. Retrieved January 2018, from http://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/foodborne-illness-and-disease/salmonella/sap-one-year

Food Safety and Inspection Service (FSIS), United States Department of Agriculture (USDA). (2017). *Serotypes profile of Salmonella isolates from meat and poultry products, January 1998 through December 2011*. Retrieved February 2018, from https://www.fsis.usda.gov/wps/

wcm/connect/3866026a-582d-4f0e-a8ce-851b39c7390f/Salmonella-Serotype-Annual-2014.pdf?MOD=AJPERES

Founou, L. L., Founou, R. C., & Essack, S. Y. (2016). Antibiotic resistance in the food chain: A developing country-perspective. *Frontiers in Microbiology, 7*, 1881.

Furtula, V., Jackson, C. R., Farrell, E. G., Barrett, J. B., Hiott, L. M., & Chambers, P. A. (2013). Antimicrobial resistance in *Enterococcus* spp. isolated from environmental samples in an area of intensive poultry production. *International Journal of Environmental Research and Public Health, 10*(3), 1020–1036.

Gao, L., Hu, J., Zhang, X., Ma, R., Gao, J., Li, S., et al. (2014). Dissemination of ESBL-producing *Escherichia coli* of chicken origin to the nearby river water. *Journal of Molecular Microbiology and Biotechnology, 24*(4), 279–285.

Gao, R., Hu, Y., Li, Z., Sun, J., Wang, Q., Lin, J., et al. (2016). Dissemination and mechanism for the *MCR*-1 colistin resistance. *PLoS Pathogens, 12*(11), e1005957.

Grimont, P. A., & Weill, F. X. (2007). Antigenic formulae of the *Salmonella* serovars. *WHO Collaborating Centre for Reference and Research on Salmonella, 9*, 110–166.

Guenther, S., Falgenhauer, L., Semmler, T., Imirzalioglu, C., Chakraborty, T., Roesler, U., et al. (2017). Environmental emission of multiresistant *Escherichia coli* carrying the colistin resistance gene *mcr*-1 from German swine farms. *Journal of Antimicrobial Chemotherapy, 72*(5), 1289–1292.

Guibourdenche, M., Roggentin, P., Mikoleit, M., Fields, P. I., Bockemühl, J., Grimont, P. A., et al. (2010). Supplement 2003–2007 (No. 47) to the white-Kauffmann-Le minor scheme. *Research in Microbiology, 161*(1), 26–29.

Helmuth, R. (2000). Antibiotic resistance in *Salmonella*. In C. Wray & A. Wray (Eds.), *Salmonella in domestic animals* (Vol. 1, pp. 89–106). New York: CABI Publishing.

Hembach, N., Schmid, F., Alexander, J., Hiller, C., Rogall, E. T., & Schwartz, T. (2017). Occurrence of the *mcr*-1 colistin resistance gene and other clinically relevant antibiotic resistance genes in microbial populations at different municipal wastewater treatment plants in Germany. *Frontiers in Microbiology, 8*, 1282–1292.

Hribar, C., & Schultz, M. (2010). *Understanding concentrated animal feeding operations and their impact on communities*. Bowling Green, OH: National Association of Local Boards of Health. Retrieved February, 18, 2013.

Hruby, C. E., Soupir, M. L., Moorman, T. B., Pederson, C., & Kanwar, R. (2018). *Salmonella* and fecal indicator bacteria survival in soils amended with poultry manure. *Water, Air, & Soil Pollution, 229*(2), 32–40.

Humphrey, T. J., Jørgensen, F., Frost, J. A., Wadda, H., Domingue, G., Elviss, N. C., et al. (2005). Prevalence and subtypes of ciprofloxacin-resistant *Campylobacter* spp. in commercial poultry flocks before, during, and after treatment with fluoroquinolones. *Antimicrobial Agents and Chemotherapy, 49*(2), 690–698.

Hur, J., Jawale, C., & Lee, J. H. (2012). Antimicrobial resistance of *Salmonella* isolated from food animals: A review. *Food Research International, 45*(2), 819–830.

Interagency Food Safety Analytics Collaboration (IFSAC) Project. (2015). *Estimates for Salmonella, Escherichia coli O157 (E. coli O157), Listeria monocytogenes (Lm), and Campylobacter using Outbreak Surveillance Data*. Retrieved February 2018, from http://www.cdc.gov/foodsafety/pdfs/ifsac-project-report-508c.pdf

Jackson, B. R., Griffin, P. M., Cole, D., Walsh, K. A., & Chai, S. J. (2013). Outbreak-associated *Salmonella enterica* serotypes and food commodities, United States, 1998–2008. *Emerging Infectious Diseases, 19*(8), 1239–1244.

Keelara, S., Scott, H. M., Morrow, W. M., Gebreyes, W. A., Correa, M., Nayak, R., et al. (2013). Longitudinal study of distributions of similar antimicrobial-resistant *Salmonella* serovars in pigs and their environment in two distinct swine production systems. *Applied and Environmental Microbiology, 79*(17), 5167–5178.

Kniel, K. E., Kumar, D., & Thakur, S. (2018). Understanding the complexities of food safety using a "one health" approach. *Microbiology Spectrum, 6*(1), PFS-0021-2017.

Kumar, D., Pornsukarom, S., Sivaraman, G. K., & Thakur, S. (2018). Environmental dissemination of multidrug methicillin-resistant *Staphylococcus sciuri* after application of manure from commercial swine production systems. *Foodborne Pathogens and Disease, 15*(4), 210–217.

Landers, T. F., Cohen, B., Wittum, T. E., & Larson, E. L. (2012). A review of antibiotic use in food animals: Perspective, policy, and potential. *Public Health Reports, 127*(1), 4–22.

Lee, M. D., Sanchez, S., Zimmer, M., Idris, U., Berrang, M. E., & McDermott, P. F. (2002). Class 1 integron-associated tobramycin-gentamicin resistance in *Campylobacter jejuni* isolated from the broiler chicken house environment. *Antimicrobial Agents and Chemotherapy, 46*(11), 3660–3664.

Lertworapreecha, M., Sutthimusik, S., & Tontikapong, K. (2012). Antimicrobial resistance in *Salmonella enterica* isolated from pork, chicken, and vegetables in southern Thailand. *Jundishapur Journal of Microbiology, 6*(1), 36–41.

Levy, S. B., & Marshall, B. (2004). Antibacterial resistance worldwide: Causes, challenges and responses. *Nature Medicine, 10*(12 Suppl), S122.

Li, R., Lai, J., Wang, Y., Liu, S., Li, Y., Liu, K., et al. (2013). Prevalence and characterization of *Salmonella* species isolated from pigs, ducks and chickens in Sichuan Province, China. *International Journal of Food Microbiology, 163*(1), 14–18.

Liljebjelke, K. A., Hofacre, C. L., White, D. G., Ayers, S., Lee, M. D., & Maurer, J. J. (2017). Diversity of antimicrobial resistance phenotypes in *Salmonella* isolated from commercial poultry farms. *Frontiers in Veterinary Science, 4*, 96–104.

Liu, Y. Y., Wang, Y., Walsh, T. R., Yi, L. X., Zhang, R., Spencer, J., et al. (2016). Emergence of plasmid-mediated colistin resistance mechanism *MCR*-1 in animals and human beings in China: A microbiological and molecular biological study. *The Lancet Infectious Diseases, 16*(2), 161–168.

Marti, R., Scott, A., Tien, Y. C., Murray, R., Sabourin, L., Zhang, Y., et al. (2013). Impact of manure fertilization on the abundance of antibiotic-resistant bacteria and frequency of detection of antibiotic resistance genes in soil and on vegetables at harvest. *Applied and Environmental Microbiology, 79*(18), 5701–5709.

Mattiello, S. P., Drescher, G., Barth, V. C., Ferreira, C. A., & Oliveira, S. D. (2015). Characterization of antimicrobial resistance in *Salmonella enterica* strains isolated from Brazilian poultry production. *Antonie Van Leeuwenhoek, 108*(5), 1227–1238.

McCollister, B., Kotter, C. V., Frank, D. N., Washburn, T., & Jobling, M. G. (2016). Whole-genome sequencing identifies in vivo acquisition of a *bla*CTX-M-27-carrying IncFII transmissible plasmid as the cause of ceftriaxone treatment failure for an invasive *Salmonella enterica* serovar typhimurium infection. *Antimicrobial Agents and Chemotherapy, 60*(12), 7224–7235.

McDermott, P. F., Bodeis, S. M., English, L. L., White, D. G., Walker, R. D., Zhao, S., et al. (2002). Ciprofloxacin resistance in *Campylobacter jejuni* evolves rapidly in chickens treated with fluoroquinolones. *The Journal of Infectious Diseases, 185*(6), 837–840.

Mollenkopf, D. F., Stull, J. W., Mathys, D. A., Bowman, A. S., Feicht, S. M., Grooters, S. V., et al. (2017). Carbapenemase-producing *Enterobacteriaceae* recovered from the environment of a swine farrow-to-finish operation in the United States. *Antimicrobial Agents and Chemotherapy, 61*(2), e01298–e01216.

Mulvey, M. R., Boyd, D. A., Olson, A. B., Doublet, B., & Cloeckaert, A. (2006). The genetics of *Salmonella* genomic island 1. *Microbes and Infection, 8*(7), 1915–1922.

Nandi, S., Maurer, J. J., Hofacre, C., & Summers, A. O. (2004). Gram-positive bacteria are a major reservoir of Class 1 antibiotic resistance integrons in poultry litter. *Proceedings of the National Academy of Sciences, 101*(18), 7118–7122.

Nhung, N. T., Cuong, N. V., Thwaites, G., & Carrique-Mas, J. (2016). Antimicrobial usage and antimicrobial resistance in animal production in Southeast Asia: A review. *Antibiotics, 5*(4), 37–45.

Ovejero, C. M., Delgado-Blas, J. F., Calero-Caceres, W., Muniesa, M., & Gonzalez-Zorn, B. (2017). Spread of *mcr*-1-carrying *Enterobacteriaceae* in sewage water from Spain. *Journal of Antimicrobial Chemotherapy, 72*(4), 1050–1053.

Padungtod, P., & Kaneene, J. B. (2006). *Salmonella* in food animals and humans in northern Thailand. *International Journal of Food Microbiology, 108*(3), 346–354.

Papadopoulos, T., Petridou, E., Zdragas, A., Nair, S., Peters, T., de Pinna, E., et al. (2015). Phenotypic and molecular characterization of multidrug-resistant *Salmonella enterica* serovar Hadar in Greece, from 2007 to 2010. *Clinical Microbiology and Infection, 21*(2), 149–1e1.

Pornsukarom, S., & Thakur, S. (2017). Horizontal dissemination of antimicrobial resistance determinants in multiple *Salmonella* serotypes following isolation from the commercial swine operation environment after manure application. *Applied and Environmental Microbiology, 83*(20), e01503–e01517.

Rinsky, J. L., Nadimpalli, M., Wing, S., Hall, D., Baron, D., Price, L. B., et al. (2013). Livestock-associated methicillin and multidrug resistant *Staphylococcus aureus* is present among industrial, not antibiotic-free livestock operation workers in North Carolina. *PLoS One, 8*(7), e67641.

Roberts, R. R., Hota, B., Ahmad, I., Scott, R. D., Foster, S. D., Abbasi, F., et al. (2009). Hospital and societal costs of antimicrobial-resistant infections in a Chicago teaching hospital: Implications for antibiotic stewardship. *Clinical Infectious Diseases, 49*(8), 1175–1184.

Rossi, F., Girardello, R., Morais, C., Cury, A. P., Martins, L. F., da Silva, A. M., et al. (2017). Plasmid-mediated *mcr*-1 in carbapenem-susceptible *Escherichia coli* ST156 causing a blood infection: An unnoticeable spread of colistin resistance in Brazil? *Clinics, 72*(10), 642–644.

Rouger, A., Tresse, O., & Zagorec, M. (2017). Bacterial contaminants of poultry meat: Sources, species, and dynamics. *Microorganisms, 5*(3), 50–58.

Shah, D. H., Paul, N. C., Sischo, W. C., Crespo, R., & Guard, J. (2017). Population dynamics and antimicrobial resistance of the most prevalent poultry-associated *Salmonella* serotypes. *Poultry Science, 96*(3), 687–702.

Sims, L. D. (2008, November). Risks associated with poultry production systems. In *International conference poultry in the twenty-first century* (pp. 19–23).

Singer, R. S., & Hofacre, C. L. (2006). Potential impacts of antibiotic use in poultry production. *Avian Diseases, 50*(2), 161–172.

Sinwat, N., Angkittitrakul, S., & Chuanchuen, R. (2015). Characterization of antimicrobial resistance in *Salmonella enterica* isolated from pork, chicken meat, and humans in Northeastern Thailand. *Foodborne Pathogens and Disease, 12*(9), 759–765.

Skariyachan, S., Setlur, A. S., & Naik, S. Y. (2016). Evolution and prevalence of multidrug resistance among foodborne pathogens. In *Food borne pathogens and antibiotic resistance*. Hoboken, NJ: Wiley.

Smith, K. E., Besser, J. M., Hedberg, C. W., Leano, F. T., Bender, J. B., Wicklund, J. H., et al. (1999). Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992–1998. *New England Journal of Medicine, 340*(20), 1525–1532.

Thai, T. H., & Yamaguchi, R. (2012). Molecular characterization of antibiotic-resistant *Salmonella* isolates from retail meat from markets in Northern Vietnam. *Journal of Food Protection, 75*(9), 1709–1714.

Thai, T. H., Hirai, T., Lan, N. T., & Yamaguchi, R. (2012). Antibiotic resistance profiles of *Salmonella* serovars isolated from retail pork and chicken meat in North Vietnam. *International Journal of Food Microbiology, 156*(2), 147–151.

Thanner, S., Drissner, D., & Walsh, F. (2016). Antimicrobial resistance in agriculture. *MBio, 7*(2), e02227–e02215.

The Pew Charitable Trusts. (2013). *The business of broilers: Hidden costs of putting a chicken on every grill*. Retrieved March 2018, from http://www.pewtrusts.org/en/research-and-analysis/reports/2013/12/20/the-business-of-broilers-hidden-costs-of-putting-a-chicken-on-every-grill

Unicomb, L., Ferguson, J., Riley, T. V., & Collignon, P. (2003). Fluoroquinolone resistance in *Campylobacter* absent from isolates, Australia. *Emerging Infectious Diseases, 9*(11), 1482–1483.

Van Boeckel, T. P., Gandra, S., Ashok, A., Caudron, Q., Grenfell, B. T., Levin, S. A., et al. (2014). Global antibiotic consumption 2000 to 2010: An analysis of national pharmaceutical sales data. *The Lancet Infectious Diseases, 14*(8), 742–750.

Van Boeckel, T. P., Brower, C., Gilbert, M., Grenfell, B. T., Levin, S. A., Robinson, T. P., et al. (2015). Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences, 112*(18), 5649–5654.

Van, T. T. H., Nguyen, H. N. K., Smooker, P. M., & Coloe, P. J. (2012). The antibiotic resistance characteristics of non-typhoidal *Salmonella enterica* isolated from food-producing animals, retail meat and humans in South East Asia. *International Journal of Food Microbiology, 154*(3), 98–106.

Ventola, C. L. (2015). The antibiotic resistance crisis: Part 1: Causes and threats. *Pharmacy and Therapeutics, 40*(4), 277–283.

von Wintersdorff, C. J., Penders, J., van Niekerk, J. M., Mills, N. D., Majumder, S., van Alphen, L. B., et al. (2016). Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Frontiers in Microbiology, 7*, 173.

Walsh, C. (2010). *The problem of antimicrobial resistance in the food chain*. Retrieved April 2018, from https://arrow.dit.ie/cgi/viewcontent.cgi?article=1007&context=schfsehrep

Wang, R., Dorp, L., Shaw, L. P., Bradley, P., Wang, Q., Wang, X., et al. (2018). The global distribution and spread of the mobilized colistin resistance gene *mcr*-1. *Nature Communications, 9*(1), 1179. https://doi.org/10.1038/s41467-018-03205-z

Warnes, S. L., Highmore, C. J., & Keevil, C. W. (2012). Horizontal transfer of antibiotic resistance genes on abiotic touch surfaces: Implications for public health. *MBio, 3*(6), e00489–e00412.

Wellington, E. M., Boxall, A. B., Cross, P., Feil, E. J., Gaze, W. H., Hawkey, P. M., et al. (2013). The role of the natural environment in the emergence of antibiotic resistance in gram-negative bacteria. *The Lancet Infectious Diseases, 13*(2), 155–165.

White, D. G., Zhao, S., Simjee, S., Wagner, D. D., & McDermott, P. F. (2002). Antimicrobial resistance of foodborne pathogens. *Microbes and Infection, 4*(4), 405–412.

World Economic Forum. (2014). *Global risks 2014, ninth edition*. Retrieved February 2018, from http://www3.weforum.org/docs/WEF_GlobalRisks_Report_2014.pdf

Yang, B., Cui, Y., Shi, C., Wang, J., Xia, X., Xi, M., et al. (2014). Counts, serotypes, and antimicrobial resistance of *Salmonella* isolates on retail raw poultry in the People's Republic of China. *Journal of Food Protection, 77*(6), 894–902.

Yang, Y. Q., Li, Y. X., Lei, C. W., Zhang, A. Y., & Wang, H. N. (2018). Novel plasmid-mediated colistin resistance gene *mcr*-7.1 in *Klebsiella pneumoniae*. *Journal of Antimicrobial Chemotherapy, 73*, 1791.

Yildirim, Y., Gonulalan, Z., Pamuk, S., & Ertas, N. (2011). Incidence and antibiotic resistance of *Salmonella* spp. on raw chicken carcasses. *Food Research International, 44*(3), 725–728.

Young, I., Rajić, A., Wilhelm, B. J., Waddell, L., Parker, S., & McEwen, S. A. (2009). Comparison of the prevalence of bacterial enteropathogens, potentially zoonotic bacteria and bacterial resistance to antimicrobials in organic and conventional poultry, swine and beef production: A systematic review and meta-analysis. *Epidemiology and Infection, 137*(9), 1217–1232.

Zhu, Y. G., Johnson, T. A., Su, J. Q., Qiao, M., Guo, G. X., Stedtfeld, R. D., et al. (2013). Diverse and abundant antibiotic resistance genes in Chinese swine farms. *Proceedings of the National Academy of Sciences, 110*(9), 3435–3440.

Zurfuh, K., Poirel, L., Nordmann, P., Nüesch-Inderbinen, M., Hächler, H., & Stephan, R. (2016). Occurrence of the plasmid-borne *mcr*-1 colistin resistance gene in extended-spectrum-β-lactamase-producing *Enterobacteriaceae* in river water and imported vegetable samples in Switzerland. *Antimicrobial Agents and Chemotherapy, 60*(4), 2594–2595.

Chapter 4
Natural and Environmentally Friendly Strategies for Controlling *Campylobacter jejuni* Colonization in Poultry, Survival in Poultry Products and Infection in Humans

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4.1 Introduction

Foodborne illnesses continue to be a significant public health concern globally. In the United States, an estimated 48 million illnesses, 128,000 hospitalizations, and 3000 deaths occur annually due to consumption of contaminated food products (Scallan et al. 2011). The annual healthcare cost for treating these infections could be as high as $77 billion USD (Scharff 2012). Among the major bacterial foodborne pathogens, *Campylobacter* is the leading cause of diarrheal illness in the United States with an estimated 1.3 million cases of campylobacteriosis occurring each year (CDC 2014). Most of these cases are sporadic in nature; however, the incidence and prevalence of campylobacteriosis cases have increased in the United States in the last 10 years. A similar increase has been recorded in Europe, Australia, and other parts of the world (Kaakoush et al. 2015; Skarp et al. 2016; Amour et al. 2016).

The genus *Campylobacter* was first proposed in 1963 by Sebald and Veron (On 2001). Recent taxonomic data suggests that the genus consists of 20 species and subspecies (Fernández et al. 2008). While there are multiple *Campylobacter* species, humans are most frequently sickened by *Campylobacter jejuni* (c. 90%),

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followed by *Campylobacter coli* (c. 10%), and less frequently by *Campylobacter lari* and *Campylobacter upsaliensis* (Friedman 2000; Gillespie et al. 2003; Taboada et al. 2013). *Campylobacter* species are widely distributed in most food animals (poultry, cattle, pigs, sheep) and household pets (Pintar et al. 2015). The main route of transmission is foodborne, via contaminated meat products, contaminated drinking water, or contaminated milk. The relative contribution of each of the above sources to the overall occurrence of infections is unclear, but consumption of contaminated poultry meat is considered to be the major contributor. Chickens are the natural host of *Campylobacter*, wherein the pathogen colonizes the poultry gut (especially the ceca) in high numbers without causing any disease or loss in production (Hermans et al. 2012; Wagenaar et al. 2015). This leads to product contamination during slaughter and subsequent human infections. Up to 80% of human *Campylobacter* infections are attributed to poultry colonization (Wagenaar et al. 2013). In the human gut, *C. jejuni* colonizes the epithelial layer of the lower intestinal tract (ileum, jejunum, colon) followed by epithelial cytopathy and enteritis (Dasti et al. 2010). The onset of symptoms usually occurs 2–5 days post-infection. In most cases, the symptoms consist of severe abdominal pain, nausea, vomiting, and diarrhea. However, in a small subset of cases, campylobacteriosis could lead to more serious illnesses such as reactive arthritis, Guillain-Barre syndrome, and Miller-Fisher syndrome leading to potentially fatal polyneuropathy and paralysis (EFSA 2011; Nachamkin et al. 2008). Serotyping of *C. jejuni* isolates recovered from Guillain-Barre cases suggests that O:19 is the predominant serotype associated with the syndrome (Kuroki et al. 1993).

The currently employed therapy for the treatment of *Campylobacter* infections includes antibiotics such as macrolides (erythromycin, clarithromycin) and fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin) (Blaser and Engberg 2008); however there are increasing reports of development of resistance to these drugs (Engberg et al. 2001; Hampton 2013; Luangtongkum et al. 2009; Olkkola et al. 2016), and several resistance-determining regions have been identified in *Campylobacter* genome (Gibreel et al. 2005; Gibreel and Taylor 2006) including Thr-86-Ile mutation in GyrA that potentiates fluoroquinolone resistance in this pathogen (Tang et al. 2017). The resistance rates to fluoroquinolones rose from 17% in 1997–1999 to 25% in 2012–2014. This rise in resistance rates occurred even after the drug group was withdrawn from use in poultry in 2005 suggesting a multitude of factors driving the selection (CDC 2017).

The food industry employs a plethora of preharvest and postharvest intervention strategies for controlling *Campylobacter*, but the human infections continue to occur. This increasing antibiotic resistance in *Campylobacter* spp. and limited efficacy of currently employed containment strategies have fueled significant interest in exploring the potential of novel approaches for controlling *Campylobacter* contamination at both preharvest and postharvest stages and combat foodborne campylobacteriosis in humans.

This chapter discusses the potential of alternative approaches (phytochemicals, probiotic bacteria, bacteriophages) for controlling *C. jejuni* in both preharvest and

postharvest stages with a focus on poultry. In addition, the efficacy of aforementioned approaches for reducing human illnesses and the potential antimicrobial mechanism(s) of action are presented.

4.2 Farm-to-Fork Approach for Controlling *Campylobacter jejuni*

In order to manage food safety risks adequately, a farm-to-fork approach is recommended that consists of controlling foodborne pathogens in reservoir animal hosts, environmental niches, and high-risk food products. This requires implementing interventions at every point of the food supply chain, from production environment to processing areas, distribution centers, storage facilities, retail food service establishment, and finally good food handling practices at home.

4.2.1 Control of Campylobacter jejuni *in Animal Reservoirs and Environment*

It is well-accepted that animal reservoirs, in particular poultry, are the primary risk factor for campylobacteriosis in humans. It has been predicted that a 1 log reduction in *Campylobacter* gut colonization in broilers could result in ~48% reduction in risk of human infection (Romero-Barrios et al. 2013). Also, a reduction in flock prevalence by 2 logs would result in approximately 30-fold reduction in incidence of campylobacteriosis (Rosenquist et al. 2003). Therefore, significant efforts are being made for developing effective intervention strategies for controlling pathogen colonization in chickens (Park et al. 2016; Ricke 2015) including *Campylobacter* spp.

Relatively less is known about the contribution of natural environment (soil, water) in the survival and/or transmission of *C. jejuni* to humans. Increasing body of evidence from genotyping studies (Ahmed et al. 2012; Nielsen et al. 2000; Wassenaar and Newell 2000), whole-genome sequencing data, and RNA-Seq suggests that *C. jejuni* strains vary in their ability to survive in the external environment. This variability stems from differences in survival strategies such as aerotolerance (Oh et al. 2017), nutritional and metabolic adaptations (Haddad et al. 2009), viable but nonculturable state (Magajna and Schraft 2015; Murphy et al. 2006), microbial commensalism (Hilbert et al. 2010; Trigui et al. 2016), and biofilm formation (Joshua et al. 2006; Reeser et al. 2007; Reuter et al. 2010). Several researchers are using this knowledge to develop intervention strategies for controlling *C. jejuni* in the processing plant and on food products (Lin 2009; Newell et al. 2011; Bronowski et al. 2014). Studies investigating the potential of phytochemicals, probiotics, and bacteriophages are presented in this chapter (Table 4.1).

Table 4.1 Studies illustrating the efficacy of (a) phytochemicals, (b) probiotics, and (c) bacteriophages in reducing *C. jejuni* at various stages in food supply chain

Farm-to-fork intervention	Phytochemicals	Source	Reference
(a) *Phytochemicals*			
Decreasing poultry colonization	Beta-resorcylic acid	Berries, Brazilian wood	Wagle et al. (2017a, b)
	Trans-cinnamaldehyde nanoemulsion	Cinnamon bark	Upadhyay et al. (2017a)
Reducing environmental persistence	Evodiamine, rutaecarpine, and evocarpine	*Euodia ruticarpa* fruit	Bezek et al. (2016)
	2(5H)-furanone, epigallocatechin gallate	*Delisea pulchra* algae, green tea	Castillo et al. (2015)
	Trans-cinnamaldehyde, carvacrol, eugenol	Cinnamon bark Oregano oil	Wagle et al. (2017a)
Lowering survival in food products	Carvacrol	Oregano oil	Shrestha et al. (2017)
	Eugenol	Clove oil	Wagle et al. (2017a, b)
	Beta-resorcylic acid	Berries, Brazilian wood	Wagle et al. (2017a, b)
	Trans-cinnamaldehyde nanoemulsion	Cinnamon bark	Upadhyay et al. (2017b)
Modulating pathogen virulence for reducing human infection risk	Trans-cinnamaldehyde, carvacrol, eugenol	Cinnamon bark Oregano oil Clove oil	Upadhyay et al. (2017a, b)
	Carvacrol	Oregano oil	Van Alphen et al. (2012)
	Clove oil		Kovács et al. (2016)
	Citrus limon, *Citrus medica*, *Citrus aurantium*		Castillo et al. (2014)

(continued)

Table 4.1 (continued)

Farm-to-fork intervention	Phytochemicals	Source	Reference
(b) *Probiotic bacteria*			
Decreasing poultry colonization	Calsporin (monospecies probiotic based on *Bacillus subtilis* C-3102), PoultryStar (multi-species probiotic containing *Enterococcus faecium*, *Pediococcus acidilactici*, *Bifidobacterium animalis*, *Lactobacillus salivarius*, and *Lactobacillus reuteri*)	–	Meunier et al. (2016)
	Lactobacillus acidophilus, *Streptococcus faecium*	–	Morishita et al. (1997)
Reducing environmental persistence	No studies	–	–
Lowering survival in food products	No studies	–	–
Modulating pathogen virulence for reducing human infection risk	*Lactobacillus gasseri* SBT2055	–	Nishiyama et al. (2014)
	Lactobacillus rhamnosus CNCM-I-3698 and *Lactobacillus farciminis* CNCM-I-3699	–	Tareb et al. (2013)
	Lactobacillus rhamnosus R0011, *Lactobacillus helveticus* R0052, *Lactobacillus salivarius* AH102, *Bifidobacterium longum* AH1205	–	Alemka et al. (2010)
	Lactobacillus helveticus strain R0052	–	Wine et al. (2009)
	Lactobacilli and bifidobacteria	–	Wagner et al. (2009)
	Lactobacillus johnsonii	–	Bereswill et al. (2017)
	Probiotic combinations (*Streptococcus thermophilus*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, and *Lactobacillus delbrueckii* ssp. *bulgaricus*)	–	Ekmekciu et al. (2017)

(continued)

Table 4.1 (continued)

Farm-to-fork intervention	Phytochemicals	Source	Reference
(c) *Probiotic bacteria*			
Decreasing poultry colonization	CP8, CP34	–	Loc-Carrillo et al. (2005)
	Bacteriophage 69 (NCTC 12669), bacteriophage 71 (NCTC 12671)	–	Wagenaar et al. (2005)
	Group II *Campylobacter* bacteriophage	–	El-Shibiny et al. (2009)
	Campylobacter phage phiCcoIBB35, phiCcoIBB37, phiCcoIBB12	–	Carvalho et al. (2010)
Reducing environmental persistence	CP8, CP30	–	Siringan et al. (2011)
Lowering survival in food products	*Campylobacter jejuni* typing phage 12,673	–	Goode et al. (2003)
	Bacteriophage φ2 (NCTC 12674)	–	Atterbury et al. (2003a)
	Campylobacter phage	–	Bigwood et al. (2008)
	Campylobacter phage NCTC12684 (group II) CP81 (group III)	–	Orquera et al. (2012)
Modulating pathogen virulence for reducing human infection risk	No studies have investigated bacteriophage therapy for reducing human campylobacteriosis	–	–

4.2.2 Control of Campylobacter jejuni *Virulence in Humans*

Advances in genomics and molecular biology have facilitated an increase in understanding of bacterial virulence, pathogenesis, and cell-to-cell signaling that a pathogen employs to cause disease. Interference with bacterial virulence and/or cell-to-cell communication is a novel approach for controlling pathogens. This approach does not kill the pathogen thereby imposing less selective pressure for the development of drug resistance than traditional strategies, which are aimed at inactivating pathogens. Intestinal colonization is the first critical step in the pathogenesis of *C. jejuni* infection which is followed by cytolethal distending toxin-mediated cytopathy and enteritis (Dasti et al. 2010). Reducing C. *jejuni* adhesion, invasion, and translocation of intestinal epithelial cells could potentially control campylobacteriosis in humans. An array of virulence factors critical for gut colonization and survival in humans have been identified (Hermans et al. 2011b). These include chemotaxis and motility systems, colonization proteins, toxin production, and cell-to-cell communication (Young et al. 2007). Very few studies have investigated the effect of natural approaches on critical virulence attributes of *C. jejuni* which are discussed below.

4.3 Applications of Phytochemicals for Controlling *Campylobacter jejuni*

Phytochemicals have been used as natural preservatives, flavor enhancers, and dietary supplements in many cultures. Similarly, food products containing beneficial microbes have been an integral part of traditional diets since ancient times. The majority of phytochemicals are secondary metabolites and are produced as a result of the interactions between plants and their immediate environment (Reichling 2010). Biochemical investigations suggest that these secondary metabolites do not contribute to any major metabolic processes in plants but potentially strengthen plant immunity and ability to survive environmental stresses (Harborne 1993) and microbial infections (Kennedy and Wightman 2011). The major groups of phytochemicals that have been used in food safety research in the last two decades include polyphenols, flavonoids, alkaloids, lectins, and tannins (Burt 2004). Some of the phytochemicals that possess significant antimicrobial efficacy include trans-cinnamaldehyde (extracted from cinnamon bark), eugenol (from clove oil), thymol, carvacrol (components in oregano oil), caprylic acid (medium-chain fatty acid from coconut oil), and beta-resorcylic acid (from Brazilian wood and berries). In addition, extracts from lemon grass (Moore-Neibel et al. 2012), turmeric (Gul and Bakht 2015), and ginger (Thongson et al. 2004) have also been investigated for their antimicrobial efficacy.

The majority of phytochemicals exert an antimicrobial effect by damaging bacterial cell wall and membrane integrity, thereby leading to leakage of cellular contents and cytopathy (Burt 2004). Moreover, recent research has revealed that phytochemicals can also modulate microbial virulence in bacterial (Azizkhani et al. 2013; Qiu et al. 2011; Upadhyay et al. 2012, 2017a, b; Wagle et al. 2017a) and fungal pathogens (Anjorin et al. 2013; Yin et al. 2015). Many phytochemicals with significant antimicrobial efficacies are also classified as GRAS (generally recognized as safe) by the FDA (FDA 2012, 2013) with low cytotoxicity and quick environmental biodegradability (Isman 2000), thus making them safe and effective antimicrobials. The selection of phytochemicals for their use as feed additives for controlling pathogens in food animals or as antimicrobials in different foods depends upon various factors that modulate their antimicrobial efficacy. Physicochemical properties such as pH, hydrophobicity, stability, and solubility affect efficacy in the host gut and environment (Negi 2012). Similarly, the presence of fat (Cava-Roda et al. 2012), sugars (Gutierrez et al. 2008), and proteins (Hyldgaard et al. 2012) modulates the antimicrobial efficacy of phytochemicals in foods. In addition, extrinsic factors such as temperature, water activity, and atmospheric composition exert a significant impact on the antimicrobial property of phytochemicals (Ncube et al. 2012) and should be considered while selecting phytochemicals for a food product. Many of the well-characterized phytochemicals have been adopted as feed additives in the poultry industry to replace antibiotic growth promoters. Essential oils are at the crux of Cargill's comprehensive approach to reduce antibiotic usage in poultry, and as many as 77 in vitro and in vivo trials have been conducted to select for the most effective essential oils, probiotics, and medium-chain fatty acids (Cargill News

Report 2016). Compounds such as lauric acid (WO 2012021306 A1), benzoic acid, thymol, eugenol, and piperine (US 8980335 B2) have been patented for use as growth promoter in poultry feed. Many essential oils are permitted for use in organic poultry under the National Organic Program and address poultry health, safety, and disease concerns in organic production systems (Donoghue et al. 2015).

4.3.1 *Phytochemicals as Antimicrobial Feed Additives for Reducing* C. jejuni *Colonization*

There has been an increase in consumer preference toward natural products during recent times. Subsequently many researchers focused on using plant-based products as potential alternatives to antibiotics in food animals (Gauthier 2003; Upadhyaya et al. 2015). Extensive research has been done on phytochemicals, and their efficacy against common foodborne pathogens has been demonstrated in vitro (Kollanoor Johny et al. 2010; Upadhyay et al. 2012). In this regard, the use of phytochemicals with proven antimicrobial efficacy appears to be a promising strategy to control pathogens in poultry production. However, factors such as chemical composition, concentration of active compound, and pH may influence the in vivo efficacy of phytochemicals (Gauthier 2003; Santiesteban-López et al. 2007; Si et al. 2006; Tassou et al. 1995). Among the various phytochemicals, previous research has demonstrated that phytochemicals such as eugenol and trans-cinnamaldehyde showed a significant reduction in *Salmonella* colonization in broiler chickens (Kollanoor-Johny et al. 2012). However the same compounds, eugenol and trans-cinnamaldehyde, did not demonstrate a similar reduction in *Campylobacter* colonization in broiler chickens (Hermans et al. 2011a). Studies conducted with other plant extracts such as thymol, carvacrol, and cranberry did not demonstrate significant reductions in *Campylobacter* colonization (Arsi et al. 2014; Woo-Ming et al. 2016). It is possible that these compounds are absorbed before reaching the ceca, dietary, or gut interactions or even the presence of other enteric microbiota may influence the in vivo efficacy of these compounds against *Campylobacter* colonization in chickens (Arsi et al. 2014). Even though plant-derived compounds showed promising results in inhibiting *Campylobacter* in vitro, further studies are needed to develop an ideal dose delivery system of an appropriate compound to produce significant reduction in *Campylobacter* in broiler chickens.

4.3.2 *Phytochemicals for Controlling Environmental Persistence and Product Contamination by* C. jejuni

Chemical sanitizers such as chlorine, quaternary ammonium compounds, and peracetic acid have been tested for their efficacy in reducing *C. jejuni* biofilms (Trachoo and Frank 2002). While *C. jejuni* in biofilms was susceptible to all sanitizers, it was

not completely eradicated by the treatments. The use of phytochemicals as natural disinfectants is a viable alternative approach to reduce *C. jejuni* persistence in the environment. Bezek et al. (2016) investigated the efficacy of *Euodia ruticarpa* fruit ethanol solution extracts for antiadhesion, antibiofilm, and antiquorum sensing activity against *C. jejuni.* The phytochemical analysis revealed that the major compounds present in the extract were evodiamine, rutaecarpine, and evocarpine. Subinhibitory concentrations of all preparations inhibited *C. jejuni* adhesion and biofilm formation with the most visible effect of the quinolinone fraction. A mutant strain that lacks CmeB efflux pump protein was the most susceptible. In another study, Castillo et al. (2015) tested the antibiofilm activity of 2(5H)-furanone and epigallocatechin gallate (polyphenolic compounds from *Delisea pulchra* algae and green tea). Results revealed that the two compounds disturbed quorum sensing activity and reduced motility and biofilm formation in *C. jejuni.* We recently reported that phytochemicals such as trans-cinnamaldehyde, carvacrol, and eugenol inhibited biofilm formation in *C. jejuni* and rapidly inactivated mature biofilm of the pathogen on polystyrene and steel surfaces (Wagle et al. 2017a). Follow-up mechanistic analysis using real-time quantitative PCR revealed that the genes coding for motility (*flaA*, *flaB*) were downregulated by all plant compounds. Eugenol also downregulated quorum sensing gene *luxS.* Scanning electron microscopy-based analysis of *C. jejuni* biofilm architecture showed that the essential oils removed exopolysaccharide from mature biofilm (Wagle et al. 2017a).

Phytochemicals are good candidates for improving the safety and shelf life of poultry products (Venkitanarayanan et al. 2013). We have screened several phytochemicals for their efficacy in reducing *C. jejuni* at postharvest stages. Some of the plant compounds such as carvacrol (Shrestha et al. 2017), eugenol (Wagle et al. 2016), and beta-resorcylic acid (Wagle et al. 2017b) are found to be very effective in reducing *C. jejuni* on chicken skin and chicken wings either as antimicrobial wash or coating treatments with chitosan. Still, significant challenges exist for application of essential oils in foods. Some of the major concerns include their poor solubility, high volatility, and organoleptic changes in the food products. To overcome these limitations, several researchers are investigating the potential of nanotechnology to improve the solubility, delivery, and antimicrobial efficacy of phytochemicals in foods (Bilia et al. 2014). The National Nanotechnology Initiative in the United States defines nanotechnology as the understanding and control of matter at a nanoscale where unique phenomena enable novel applications. This rapidly expanding sector is recognized by the European Commission as one of its six "Key Enabling Technologies" that contribute to sustainability, competitiveness, and growth in agriculture (Parisi et al. 2015). The US Food and Drug Administration (FDA) has issued a draft guidance for the use of nanomaterials in animal feed (FDA 2015). However, more research is needed to delineate the impact of food nanotechnology on human health. Some tests for nanomaterial safety have been reported (Handy and Shaw 2007); however, no internationally accepted standards for toxicity testing in food are currently available except for protocols by the US National Research Council and International Alliance for Nano Environment, Human Health and Safety Harmonization (Maynard et al. 2006; National Research Council 2007).

We have investigated the efficacy of phytochemical nanoemulsions for controlling *C. jejuni* on poultry products (Upadhyay et al. 2017b). Results suggest that nanoemulsions of trans-cinnamaldehyde are more effective (as compared to non-nanoemulsion form) in reducing *C. jejuni* counts on chicken skin and chicken wings without affecting the color of the product. Studies investigating the potential of carvacrol and eugenol nanoemulsions for controlling *C. jejuni* on chicken skin and wings are currently underway.

4.3.3 Phytochemicals as Food Supplement for Controlling Human Infections

The human gut harbors a large and complex microbial community that comprises a biomass of 1.5–2 kg, dominated by strictly anaerobic bacteria (Qin et al. 2010). Changes in diet have been linked with changes in the composition and diversity of gut microbial population (Duncan et al. 2007), which in turn affects the host's immune response (Brown et al. 2012) and susceptibility to gastrointestinal bacterial infections (Ghosh et al. 2011). Recent investigations have revealed that phytochemicals reduce bacterial virulence by affecting the transcription of critical genes (Li et al. 2011; Upadhyay et al. 2012) and expression of associated proteins (Qiu et al. 2010; Parsaeimehr et al. 2010). Our laboratory recently reported that subinhibitory concentrations of trans-cinnamaldehyde, carvacrol, and eugenol significantly reduced *C. jejuni* motility, attachment, invasion, and translocation of human intestinal cells and cytolethal distending toxin production in vitro (Upadhyay et al. 2017a). These compounds also reduced the transcription of some of the genes associated with aforementioned virulence traits. Van Alphen et al. (2012) studied the effect of carvacrol on flagellar biosynthesis and function using anti-flagella antiserum and motility bioassays, respectively. These researchers observed that the reduced *C. jejuni* motility by carvacrol is due to loss in flagella function; however, the flagellar biosynthesis was not affected. Kovács et al. (2016) reported that clove essential oil (*Syzygium aromaticum*) modulates the expression of virulence genes associated with flagella synthesis, PEB1, PEB4, lipopolysaccharide (LPS), and serine protease in *C. jejuni.* Loss of motility was also observed in response to clove essential oil.

Quorum sensing (population-dependent cell-to-cell communication) contributes to the expression/regulation of virulence determinants in pathogens. Several studies have investigated the potential of phytochemicals as quorum sensing inhibitors in foodborne pathogens (Koh et al. 2013; Persson et al. 2005; Upadhyay et al. 2013). However, very few investigations have studied their efficacy in modulating quorum sensing in *C. jejuni.* Castillo et al. (2014) investigated the efficacy of citrus extracts as inhibitors of quorum sensing in *C. jejuni*. Extracts from *Citrus limon*, *Citrus medica*, and *Citrus aurantium* reduced the activity of quorum sensing molecule AI-2 by ~90%. Since quorum sensing via LuxS protein plays an important role in *C. jejuni* virulence (Plummer et al. 2012), reducing quorum sensing could pave the way for developing antivirulence strategies for controlling *C. jejuni* infections in humans.

4.4 Applications of Probiotic Bacteria for Controlling *Campylobacter jejuni*

Etymologically the term probiotic is derived from the Greek word meaning "for life." Until recently the most commonly used definition of probiotics was that of Fuller: "probiotics are live microbial feed supplements which beneficially affect the host animal by improving microbial balance" (Fuller 1989). With increased understanding of their physiology, mechanism(s) of action, and benefits, the definition provided by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) is currently used which defines probiotics as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host." These benefits include efficient nutrient digestion (Sonnenburg et al. 2005), immune enhancement (Olszak et al. 2012), prevention of enteric infections (Candela et al. 2008), antiatherogenic properties, cholesterol-lowering attributes, and reduced risk of colorectal cancer (Ouwehand et al. 2005; Sanders and Marco 2010; Serban 2014). This has led to a rapid increase in demand, both for clinical applications and as functional foods for promoting overall health.

Although several species of microorganisms have been identified, the majority of probiotic bacteria belong to lactobacilli and bifidobacteria (Holzapfel et al. 1995). They are commonly called lactic acid bacteria since these microbes produce lactic acid as end product of carbohydrate fermentation. The genus *Bifidobacterium*, although employing a separate carbohydrate metabolic pathway, is also traditionally grouped under lactic acid bacteria (Felis and Dellaglio 2007). Since beneficial probiotic effects are often strain specific, identification of microbial strains is highly recommended to facilitate unequivocal characterization of probiotic products. This is achieved by "polyphasic taxonomy" which employs a combination of phenotypic tests and genotypic identification using DNA hybridization, 16S rRNA sequencing, etc. (Donelli et al. 2013). Although the physicochemical properties of good probiotic candidates depend on the area of application, some commonly desired traits include (1) acid/bile tolerance, (2) mucosal and gut colonization, (3) competitive exclusion/antimicrobial activity against pathogens, (4) successful immune modulation, and (5) bile salt hydrolase activity (Kechagia et al. 2013).

4.4.1 Probiotic Bacteria as Natural Biocontrol Agents for Reducing C. jejuni *Colonization*

Researchers have demonstrated both in vitro and in vivo efficacies of several probiotics to inhibit enteric pathogens such as *Salmonella*, *Campylobacter*, and *E. coli* (Chaveerach et al. 2004; Fooks and Gibson 2002; Santini et al. 2010). It has been proposed that probiotic bacteria obtained from the intestinal mucus layer of chickens, occupying the same niche as *Campylobacter*, can colonize and competitively inhibit *Campylobacter* colonization in chickens (Stern et al. 2001). Thus, the use of

probiotics seems to be a promising alternative for antibiotics for inhibiting or reducing *Campylobacter* colonization in broiler chickens. The probiotic genera that are most commonly tested for their capacity to reduce *C. jejuni* colonization are *Lactobacillus*, *Bacillus*, and *Enterococcus* (Thomrongsuwannakij et al. 2016). Probiotic cultures derived from poultry, such as *Lactobacillus acidophilus* and *Streptococcus faecium*, produced 70% reduction in fecal shedding of *C. jejuni* and 27% reduction in cecal colonization (Morishita et al. 1997). However, they were not able to prevent cecal colonization of *Campylobacter.* Similarly, Thomrongsuwannakij et al. (2016) observed that no significant reductions in *C. jejuni* colonization occurred when *Lactobacillus acidophilus*, *Bacillus subtilis*, and *Enterococcus faecium* were administered orally to broiler chickens. Further, competitive enhancement of probiotic isolates by selecting the isolates with enhanced motility or supplementing with prebiotics also demonstrated limited efficacy in reducing cecal *Campylobacter* counts (Aguiar et al. 2013; Arsi et al. 2015a). One possible reason for such inconsistencies could be due to failure of probiotic bacteria to survive gastric acidity and reach the lower intestine to colonize and compete with *Campylobacter* (Ding and Shah 2009). Encapsulation of isolates may overcome this problem but there is no assurance these isolates will have efficacy in the lower GI tract. To overcome this hurdle, Arsi et al. (2015b) screened bacterial isolates with efficacy against *Campylobacter*, and candidate isolates were tested for their in vivo efficacy by directly placing them in the lower intestinal tract via cloacal inoculation. This strategy demonstrated a significant reduction in cecal *Campylobacter* counts (1–3 $\log_{10}$) compared to the same isolates when administered orally. Based on the available literature, the use of probiotics as feed additives can be an effective strategy to reduce *Campylobacter* colonization in broiler chickens; however, differences in routes and frequency of administration, timing, and/or combination with other natural strategies need to be evaluated for a consistent reduction of *Campylobacter* in the ceca of broiler chickens (Arsi et al. 2015b; Saint-Cyr et al. 2016).

4.4.2 Probiotic Bacteria for Control of C. jejuni *in the Environment*

Chemical cross-talk between microbial species plays a key role in bacterial colonization and establishment of a microbial ecosystem in a niche (Simões et al. 2010). Hanning et al. (2008) suggested that *C. jejuni* might colonize preestablished biofilms of other bacteria. Several other studies have shown that *C. jejuni* is able to survive by forming mixed-species biofilms with other bacterial species (Sanders et al. 2007; Teh et al. 2010) that include *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, and *Salmonella* Agona. Antagonistic microbes that resist pathogen colonization in mixed-species biofilm have been observed against *Listeria monocytogenes* (Leriche and Carpentier 2000; Zhao et al. 2004); however no such studies have been conducted to control *C. jejuni* biofilms. Since many probiotic/antagonistic bacteria use similar compounds for cell-to-cell communication/

quorum sensing, it is possible that probiotics that inhibit *L. monocytogenes* and *Salmonella* biofilms would also be effective against *C. jejuni* biofilms. Thus, probiotics represent a promising bio-disinfection strategy to control *C. jejuni* in the environment; however further research is needed to validate their efficacy.

4.4.3 *Probiotic Bacteria as Anti-*Campylobacter *Supplements for Controlling Human Infections*

Bifidobacterium and *Lactobacillus* are among the well-characterized candidates proposed to possess potential probiotic effects (Guerin-Danan et al. 1998). Nishiyama et al. (2014) investigated the efficacy of *Lactobacillus gasseri* SBT2055 in reducing colonization of human intestinal cells (Int 407) by *C. jejuni* 81–176. Pretreatment with *L. gasseri* SBT2055 significantly reduced attachment and invasion of the pathogen on human intestinal cells. Follow-up mechanistic analysis revealed that co-aggregation phenotype mediated by the proteinaceous cell-surface components of the probiotic contributed to this effect. The anti-*Campylobacter* aggregation phenotype has also been observed with other probiotics such as *Lactobacillus rhamnosus* CNCM-I-3698 and *Lactobacillus farciminis* CNCM-I-3699 (Tareb et al. 2013). In another study, Alemka et al. (2010) investigated the efficacy of several probiotics (*L. rhamnosus* R0011, *L. helveticus* R0052, *L. salivarius* AH102, *Bifidobacterium longum* AH1205) or their combinations in reducing *C. jejuni* 81–176 colonization on mucus-producing HT29MTXE12 cell line. Results suggested that the probiotics colonized E12 mucus and bound to underlying cells, thereby attenuating *C. jejuni* attachment, internalization, and basolateral translocation across the epithelial barrier. Wine et al. (2009) had similar results with *Lactobacillus helveticus* strain R0052. These researchers observed that the probiotic bacterium was able to colonize T84 human colon cells and reduced *C. jejuni* invasion of the epithelial cells, potentially through competitive exclusion.

The anti-C*ampylobacter* efficacy of probiotics has also been tested in mammalian models. Wagner et al. (2009) investigated the efficacy of lactobacilli and bifidobacteria in resisting *C. jejuni* colonization in immunocompetent BALB/c and immunodeficient Tgepsilon26 mice. In both mice species, the probiotic bacteria were able to exclude *C. jejuni* population 7 days after challenge. In another study, Bereswill et al. (2017) tested the efficacy of *Lactobacillus johnsonii* in reducing *C. jejuni* (strain 81–176) colonization in secondary abiotic mice. Although the probiotic was not able to reduce pathogen numbers (either as prophylactic or therapeutic regimen), the probiotic treatments ameliorated intestinal, extraintestinal, and systemic pro-inflammatory immune responses (Il-6, MCP-1, TNF, nitric oxide) suggesting that such treatments could modify the outcome of the disease. Similar results were observed by Ekmekciu et al. (2017) with probiotic combination (*Streptococcus thermophilus*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, and *Lactobacillus delbrueckii* ssp. *bulgaricus*) where the

treatments were not able to reduce pathogen colonization but reduced pathogen-mediated intestinal apoptosis and pro-inflammatory immune responses. Concentrations of anti-inflammatory mediators such as IL-10 were significantly elevated in the colon in response to probiotic treatments. Taken together, these findings highlight the efficacy of some probiotic bacteria in reducing *C. jejuni* colonization or associated pathology. However, further studies are required to test more probiotic candidates and delineate the potential mechanism(s) of action in appropriate models of human campylobacteriosis.

4.5 Applications of Bacteriophages for Controlling *Campylobacter jejuni*

Bacteriophages are viruses that specifically infect bacteria and kill them by sequential internal replication and cell lysis. Phages are the most ubiquitous organisms on earth with total numbers estimated to be between 10^{30} and 10^{32}. Phages are abundant in all water bodies (Rohwer 2003), soil, plant, and animals. They are frequently isolated from drinking water (Armon et al. 1997) and from a wide range of food products (Gautier et al. 1995). They play a key role in microbial ecosystem balance and evolution of the cellular world (Sime-Ngando 2014). Since their discovery in 1915, they have been extensively studied for applications in both medical and agricultural settings, and a plethora of phages have been discovered. Phages offer several advantages as natural biocontrol agents including:

1. High specificity: This facilitates targeted removal of pathogens while leaving the beneficial or normal microbiota unaffected.
2. Self-propagating/limiting: Since bacteriophages are obligate parasites that require a host system to propagate, a single dose multiplies in response to a threshold host population eventually killing the target pathogen. Subsequently, in the absence of a host, the virus population also reduces.
3. Low toxicity: Bacteriophages consist of nucleic acid and proteins; therefore, their chances of causing toxicity to the animal/human host are relatively low.

Apart from the aforementioned attributes, bacteriophages are relatively easy to isolate and culture in laboratory conditions and can withstand food processing environmental stresses, thereby making them good candidates for use as natural, safe, and environmentally friendly biocontrol agents (Sillankorva et al. 2012).

4.5.1 Bacteriophages for Improving Preharvest Food Safety

Bacteriophages are viruses that infect and kill bacteria by attaching to the specific receptors on cell surface followed by injecting DNA into the host and taking over the host cell machinery for rapid multiplication and release by lysis of the host

bacterium (Doyle and Erickson 2006; Duckworth and Gulig 2002). Researchers have successfully demonstrated the use of phages to control pathogens such as *E. coli* (Huff et al. 2005) and *Salmonella* (Fiorentin et al. 2005; Higgins et al. 2005). The potential use of bacteriophages for reducing *Campylobacter* colonization in broiler chickens has also been studied (El-Shibiny et al. 2009; Carrillo et al. 2005; Wagenaar et al. 2005). Carrillo et al. (2005) screened 53 lytic bacteriophages against a panel of 50 *Campylobacter* isolates from both chicken and human origins and identified two phage candidates with broad host lysis. These phages, CP8 and CP34, when administered along with antacid suspension, were effective in reducing pathogen counts between 0.5 and 5 log CFU/g of cecal contents over a 5 day period post-administration. These reductions were dependent on phage-*Campylobacter* combination, dose, and time of treatment. Wagenaar et al. (2005) studied both prophylactic and therapeutic efficacies of phages in reducing *Campylobacter* colonization in broiler chickens. These studies concluded that prophylactic treatment using bacteriophages will only delay colonization but not reduce *Campylobacter* in comparison to the control. Carvalho et al. (2010) incorporated *Campylobacter*-specific bacteriophages into poultry feed and observed a 2 log reduction of *Campylobacter* when compared to control. In another study, Kittler et al. (2013) administered a bacteriophage cocktail through drinking water at 36 days of age (1–7 days before slaughter) in commercial poultry. A *C. jejuni* reduction of 3.2 log CFU/g of cecal content was observed versus the controls suggesting that a broad spectrum of bacteriophage along with judicious selection of application time could improve poultry safety. In contrast to the diversity of *Campylobacter* strains in broiler chickens, bacteriophages are highly specific, and potential development of phage resistance is possible. This diversity should be considered when evaluating microbial risk assessments and developing control strategies. Consumer acceptability further limits the use of phages for controlling *Campylobacter* in broiler chickens. Despite these hurdles, bacteriophages could be an effective tool to reduce *Campylobacter* in broiler chickens. However, further studies should be done prior to application of bacteriophages in broiler chickens to avoid possible emergence of phage-resistant *Campylobacter* strains.

4.5.2 Bacteriophages for Control of C. jejuni *in the Environment*

Siringan et al. (2011) investigated the effect of *C. jejuni*-specific bacteriophages CP8 and CP30 for controlling *C. jejuni* biofilm formed on glass at 37 °C. A reduction of 1–3 log CFU/cm^2 was observed 24 h posttreatment along with dispersal of the biofilm matrix. However, resistance to bacteriophage in planktonic cells of *C. jejuni* NCTC 11168 was also observed. Several studies have also investigated the efficacy of bacteriophages in reducing *C. jejuni* on poultry products (Atterbury et al. 2003a; Bigwood et al. 2008; Goode et al. 2003; Orquera et al. 2012; Sulakvelidze and Barrow 2005). A majority of these studies applied phages at ~4–7 log PFU/

sample at an MOI (multiplicity of infection) of 10–100 and observed a reduction of ~1–2 log CFU/sample in *C. jejuni* counts. Much work is still required to understand how best to apply phages to control *C. jejuni* in processing environment and food products. For example, combination of phage therapy with well-characterized hurdles (e.g., low temperature, dehydration, heat) should be investigated. Atterbury et al. (2003b) observed that application of *C. jejuni*-specific bacteriophage in combination with freezing was more effective in reducing pathogen counts than single treatments. In addition, optimization of phage purification technology is required to facilitate bacterial toxin-free collection of phages for application on food products (Janež and Loc-Carrillo 2013). Convective interaction media (CIM) monolithic column is one such technology that holds promise for purifying and concentrating phages (Smrekar et al. 2008).

4.5.3 Bacteriophage Therapy for Controlling Human Infections

The use of phages is a safe and effective approach for controlling bacterial infections in humans (Ho 2001; Merril et al. 2003; Sulakvelidze and Kutter 2005). The use of bacteriophages to treat bacterial infections in humans was studied in the early 1900s. However, with the discovery of antibiotics, this area of research slowed down. In recent years, with the emergence of antibiotic resistance in bacterial pathogens, research exploring the potential of bacteriophages for controlling human infections has seen a renaissance. Several studies have investigated the efficacy of bacteriophage for controlling nosocomial (Weber-Dąbrowska et al. 2001) and foodborne pathogens such as *L. monocytogenes* (Mai et al. 2010), *E. coli* O157:H7 (Capparelli et al. 2006; Maura et al. 2012; Sarker et al. 2012), and *Salmonella* spp. (Nikkhahi et al. 2017; Shin et al. 2012). However, studies investigating phage efficacy for controlling human campylobacteriosis either in vitro or in a mammalian host have not been conducted.

4.6 Conclusion and Future Directions

The use of phytochemicals, probiotics, bacteriophages, or their combinations is a promising strategy for controlling pathogenic *Campylobacter* in animal hosts, processing environment, food products, and humans. However, the effect of phytochemicals on the organoleptic properties of poultry products is a significant concern and requires more investigations. Similarly, comprehensive characterization of probiotics is recommended before commercial application. Further studies are also needed in exploring potential synergistic effects between different phytochemicals, probiotics, or bacteriophages for enhanced antimicrobial efficacy and broader applications. Moreover, delineating the mechanism(s) of action of different

phytochemical groups, probiotics, and bacteriophages is essential for developing efficient interventions against *Campylobacter* spp. With recent developments in next-generation sequencing, it is now possible to characterize the effect of various dietary interventions on the gut microbiome of food animals including chickens (Oakley et al. 2014). Several scientific groups have characterized the gut microbiome of poultry (Brisbin et al. 2008; Park et al. 2016; Sergeant et al. 2014), and more than 1000 bacterial species have been identified (Chambers and Gong 2011). Majority of bacterial species belong to *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*. Moreover, the role of various proteins and metabolites produced by gut microbiota that modulate host immune status and susceptibility to various enteric infections is also being investigated (Schroeder and Bäckhed 2016). Metagenomic- and metaproteomic-based approaches are currently being used to delineate the metabolic capabilities of microbiota. Systems microbiology that aims to study the dynamic interactions of more than one component in a biological system is a novel approach that could be used to better characterize microbial interactions, thereby developing effective intervention strategies. The effect of phytochemicals, probiotics, or bacteriophages on the epigenome of host cells is another unexplored area that could lead to development of novel therapeutics against *C. jejuni* and other foodborne pathogens.

References

Aguiar, V. F., Donoghue, A. M., Arsi, K., Reyes-Herrera, I., Metcalf, J. H., de los Santos, F. S., et al. (2013). Targeting motility properties of bacteria in the development of probiotic cultures against Campylobacter jejuni in broiler chickens. *Foodborne Pathogens and Disease, 10*(5), 435–441.

Ahmed, M. U., Dunn, L., & Ivanova, E. P. (2012). Evaluation of current molecular approaches for genotyping of *Campylobacter jejuni* strains. *Foodborne Pathogens and Disease, 9*(5), 375–385.

Alemka, A., Clyne, M., Shanahan, F., Tompkins, T., Corcionivoschi, N., & Bourke, B. (2010). Probiotic colonization of the adherent mucus layer of HT29MTXE12 cells attenuates *Campylobacter jejuni* virulence properties. *Infection and Immunity, 78*(6), 2812–2822.

Amour, C., Gratz, J., Mduma, E., Svensen, E., Rogawski, E. T., McGrath, M., et al. (2016). Epidemiology and impact of Campylobacter infection in children in 8 low-resource settings: Results from the MAL-ED study. *Clinical Infectious Diseases, 63*(9), 1171–1179.

Anjorin, T. S., Salako, E. A., & Makun, H. A. (2013). Control of toxigenic fungi and mycotoxins with phytochemicals: Potentials and challenges. In *Mycotoxin and food safety in developing countries*. Rijeka, Croatia: InTech.

Armon, R., Araujo, R., Kott, Y., Lucena, F., & Jofre, J. (1997). Bacteriophages of enteric bacteria in drinking water, comparison of their distribution in two countries. *Journal of Applied Microbiology, 83*(5), 627–633.

Arsi, K., Donoghue, A. M., Venkitanarayanan, K., Kollanoor-Johny, A., Fanatico, A. C., Blore, P. J., et al. (2014). The efficacy of the natural plant extracts, thymol and carvacrol against *Campylobacter* colonization in broiler chickens. *Journal of Food Safety, 34*, 321–325.

Arsi, K., Donoghue, A. M., Woo-Ming, A., Blore, P. J., & Donoghue, D. J. (2015a). The efficacy of selected probiotic and prebiotic combinations in reducing *Campylobacter* colonization in broiler chickens. *Journal of Applied Poultry Research, 24*(3), 327–334.

Arsi, K., Donoghue, A. M., Woo-Ming, A., Blore, P. J., & Donoghue, D. J. (2015b). Intracloacal inoculation, an effective screening method for determining the efficacy of probiotic bacterial

isolates against *Campylobacter* colonization in broiler chickens. *Journal of Food Protection, 78*(1), 209–213.

Atterbury, R. J., Connerton, P. L., Dodd, C. E., Rees, C. E., & Connerton, I. F. (2003a). Application of host-specific bacteriophages to the surface of chicken skin leads to a reduction in recovery of *Campylobacter jejuni*. *Applied and Environmental Microbiology, 69*(10), 6302–6306.

Atterbury, R. J., Connerton, P. L., Dodd, C. E., Rees, C. E., & Connerton, I. F. (2003b). Isolation and characterization of *Campylobacter* bacteriophages from retail poultry. *Applied and Environmental Microbiology, 69*(8), 4511–4518.

Azizkhani, M., Misaghi, A., Basti, A. A., Gandomi, H., & Hosseini, H. (2013). Effects of Zataria multiflora Boiss. essential oil on growth and gene expression of enterotoxins A, C and E in *Staphylococcus aureus* ATCC 29213. *International Journal of Food Microbiology, 163*(2), 159–165.

Bereswill, S., Ekmekciu, I., Escher, U., Fiebiger, U., Stingl, K., & Heimesaat, M. M. (2017). *Lactobacillus johnsonii* ameliorates intestinal, extra-intestinal and systemic pro-inflammatory immune responses following murine *Campylobacter jejuni* infection. *Scientific Reports, 7*(1), 2138.

Bezek, K., Kurinčič, M., Knauder, E., Klančnik, A., Raspor, P., Bucar, F., et al. (2016). Attenuation of adhesion, biofilm formation and quorum sensing of *Campylobacter jejuni* by *Euodia ruticarpa*. *Phytotherapy Research, 30*(9), 1527–1532.

Bigwood, T., Hudson, J. A., Billington, C., Carey-Smith, G. V., & Heinemann, J. A. (2008). Phage inactivation of foodborne pathogens on cooked and raw meat. *Food Microbiology, 25*(2), 400–406.

Bilia, A. R., Guccione, C., Isacchi, B., Righeschi, C., Firenzuoli, F., & Bergonzi, M. C. (2014). Essential oils loaded in nanosystems: A developing strategy for a successful therapeutic approach. *Evidence-based Complementary and Alternative Medicine, 2014*, 1–14.

Blaser, M. J., & Engberg, J. (2008). Clinical aspects of *Campylobacter jejuni* and *Campylobacter coli* infections. In I. Nachamkin, C. Szymanski, & M. Blaser (Eds.), *Campylobacter* (3rd ed., pp. 99–121). Washington, DC: ASM Press.

Brisbin, J. T., Gong, J., & Sharif, S. (2008). Interactions between commensal bacteria and the gut-associated immune system of the chicken. *Animal Health Research Reviews, 9*(1), 101–110.

Brown, K., DeCoffe, D., Molcan, E., & Gibson, D. L. (2012). Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. *Nutrients, 4*(8), 1095–1119.

Bronowski, C., James, C. E. and Winstanley, C., 2014. Role of environmental survival in transmission of *Campylobacter jejuni*. FEMS microbiology letters, 356(1), 8–19.

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology, 94*(3), 223–253.

Candela, M., Perna, F., Carnevali, P., Vitali, B., Ciati, R., Gionchetti, P., et al. (2008). Interaction of probiotic Lactobacillus and Bifidobacterium strains with human intestinal epithelial cells: Adhesion properties, competition against enteropathogens and modulation of IL-8 production. *International Journal of Food Microbiology, 125*(3), 286–292.

Capparelli, R., Ventimiglia, I., Roperto, S., Fenizia, D., & Iannelli, D. (2006). Selection of an Escherichia coli O157: H7 bacteriophage for persistence in the circulatory system of mice infected experimentally. *Clinical Microbiology and Infection, 12*(3), 248–253.

Cargill News Report. (2016). *Essential oils key to Cargill's comprehensive approach to reducing antibiotics in poultry*. https://www.cargill.com/news/releases/2016/NA31925706.jsp

Carrillo, C. L., Atterbury, R. J., El-Shibiny, A., Connerton, P. L., Dillon, E., Scott, A., et al. (2005). Bacteriophage therapy to reduce *Campylobacter jejuni* colonization of broiler chickens. *Applied and Environmental Microbiology, 71*(11), 6554–6563.

Carvalho, C. M., Gannon, B. W., Halfhide, D. E., Santos, S. B., Hayes, C. M., Roe, J. M., et al. (2010). The *in vivo* efficacy of two administration routes of a phage cocktail to reduce numbers of *Campylobacter coli* and *Campylobacter jejuni* in chickens. *BMC Microbiology, 10*(1), 232.

Castillo, S., Heredia, N., Arechiga-Carvajal, E., & García, S. (2014). Citrus extracts as inhibitors of quorum sensing, biofilm formation and motility of *Campylobacter jejuni*. *Food Biotechnology, 28*(2), 106–122.

Castillo, S., Heredia, N., & García, S. (2015). 2 (5H)-Furanone, epigallocatechin gallate, and a citric-based disinfectant disturb quorum-sensing activity and reduce motility and biofilm formation of *Campylobacter jejuni*. *Folia Microbiologica, 60*(1), 89–95.

Cava-Roda, R. M., Taboada-Rodríguez, A., Valverde-Franco, M. T., & Marín-Iniesta, F. (2012). Antimicrobial activity of vanillin and mixtures with cinnamon and clove essential oils in controlling *Listeria monocytogenes* and *Escherichia coli O157: H7* in milk. *Food and Bioprocess Technology, 5*(6), 2120–2131.

CDC. (2014). *Campylobacter*. Available online at: https://www.cdc.gov/foodsafety/diseases/campylobacter/

CDC. (2017). *Antibiotic resistance in campylobacter*. Available online at: https://www.cdc.gov/campylobacter/campy-antibiotic-resistance.html

Chambers, J. R., & Gong, J. (2011). The intestinal microbiota and its modulation for *Salmonella* control in chickens. *Food Research International, 44*(10), 3149–3159.

Chaveerach, P., Lipman, L. J. A., & Van Knapen, F. (2004). Antagonistic activities of several bacteria on *in vitro* growth of 10 strains of *Campylobacter jejuni/coli*. *International Journal of Food Microbiology, 90*(1), 43–50.

Dasti, J. I., Tareen, A. M., Lugert, R., Zautner, A. E., & Groß, U. (2010). *Campylobacter jejuni:* A brief overview on pathogenicity-associated factors and disease-mediating mechanisms. *International Journal of Medical Microbiology, 300*(4), 205–211.

Ding, W. K., & Shah, N. P. (2009). An improved method of microencapsulation of probiotic bacteria for their stability in acidic and bile conditions during storage. *Journal of Food Science, 74*(2), M53–M61.

Donelli, G., Vuotto, C., & Mastromarino, P. (2013). Phenotyping and genotyping are both essential to identify and classify a probiotic microorganism. *Microbial Ecology in Health and Disease, 24*(1), 20105.

Donoghue, A. M., Venkitanarayanan, K., Arsi, K., Woo-Ming, A., Upadhyaya, I., Kollanoor-Johny, A., et al. (2015). Organic poultry: Developing natural solutions for reducing pathogens and improving production. In: *Proceedings of the Organic Agricultural Conference Proceedings* (pp. 1–5).

Doyle, M. P., & Erickson, M. C. (2006). Reducing the carriage of foodborne pathogens in livestock and poultry. *Poultry Science, 85*(6), 960–973.

Duckworth, D. H., & Gulig, P. A. (2002). Bacteriophages. *BioDrugs, 16*(1), 57–62.

Duncan, S. H., Belenguer, A., Holtrop, G., Johnstone, A. M., Flint, H. J., & Lobley, G. E. (2007). Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. *Applied and Environmental Microbiology, 73*(4), 1073–1078.

EFSA. (2011). Scientific opinion on *Campylobacter* in broiler meat production: Control options and performance objectives and/or targets at different stages of the food chain. *EFSA Journal, 9*, 2105–2246.

Ekmekciu, I., von Klitzing, E., Fiebiger, U., Neumann, C., Bacher, P., Scheffold, A., et al. (2017). The probiotic compound VSL# 3 modulates mucosal, peripheral, and systemic immunity following murine broad-spectrum antibiotic treatment. *Frontiers in Cellular and Infection Microbiology, 7*, 167.

El-Shibiny, A., Scott, A., Timms, A., Metawea, Y., Connerton, P., & Connerton, I. (2009). Application of a group II *Campylobacter* bacteriophage to reduce strains of *Campylobacter jejuni* and *Campylobacter coli* colonizing broiler chickens. *Journal of Food Protection, 72*, 733–740.

Engberg, J., Aarestrup, F. M., Taylor, D. E., Gerner-Smidt, P., & Nachamkin, I. (2001). Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli:* Resistance mechanisms and trends in human isolates. *Emerging Infectious Diseases, 7*, 24–34. https://doi.org/10.3201/eid0701.010104

Felis, G. E., & Dellaglio, F. (2007). Taxonomy of lactobacilli and bifidobacteria. *Current Issues in Intestinal Microbiology, 8*(2), 44.

Fernández, H., Vera, F., Villanueva, M. P., & García, A. (2008). Occurrence of *Campylobacter* species in healthy well-nourished and malnourished children. *Brazilian Journal of Microbiology, 39*(1), 56–58.

Fiorentin, L., Vieira, N. D., & Barioni Jr., W. (2005). Oral treatment with bacteriophages reduces the concentration of *Salmonella* Enteritidis PT4 in caecal contents of broilers. *Avian Pathology, 34*(3), 258–263.

Food Drug Administration. (2012). *Code of federal regulations title 21 Part 172*. Available online at: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?CFRPart1/4172

Food Drug Administration. (2013). *Everything Added to Food in the United States (EAFUS). Doc. No. 3045–2, 4-dihydroxybenzoic acid*. Available online at: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?

Food Drug Administration. (2015). *Use of nanomaterials in food for animals*. https://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM401508.pdf

Fooks, L. J., & Gibson, G. R. (2002). Probiotics as modulators of the gut flora. *British Journal of Nutrition, 88*(Suppl 1), S39–S49.

Friedman, C. R. (2000). Epidemiology of Campylobacter jejuni infections in the United States and other industrialized nations. In *Campylobacter* (pp. 121–138). Washington, DC: ASM International.

Fuller, R. (1989). Probiotics in man and animals. *The Journal of Applied Bacteriology, 66*, 365–378.

Gauthier, R. (2003). Poultry therapeutics: New alternatives in nuevas alternativas en therapeutica aviar. In *XVIII Latin American poultry congress 2003, Bolivia*.

Gautier, M., Rouault, A., Sommer, P., & Briandet, R. (1995). Occurrence of Propionibacterium freudenreichii bacteriophages in swiss cheese. *Applied and Environmental Microbiology, 61*(7), 2572–2576.

Ghosh, S., Dai, C., Brown, K., Rajendiran, E., Makarenko, S., Baker, J., et al. (2011). Colonic microbiota alters host susceptibility to infectious colitis by modulating inflammation, redox status, and ion transporter gene expression. *American Journal of Physiology-Gastrointestinal and Liver Physiology, 301*(1), G39–G49.

Gibreel, A., Kos, V. N., Keelan, M., Trieber, C. A., Levesque, S., Michaud, S., et al. (2005). Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*: Molecular mechanism and stability of the resistance phenotype. *Journal of Antimicrobial Chemotherapy, 49*, 2753–2759. https://doi.org/10.1128/AAC.49.7.2753-2759.2005

Gibreel, A., & Taylor, D. E. (2006). Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. *Journal of Antimicrobial Chemotherapy, 58*, 243–255. https://doi.org/10.1093/jac/dkl210

Gillespie, I. A., O'Brien, S. J., Adak, G. K., Tam, C. C., Frost, J. A., Bolton, F. J., et al. (2003). Point source outbreaks of *Campylobacter jejuni* infection--are they more common than we think and what might cause them? *Epidemiology and Infection, 130*(3), 367.

Goode, D., Allen, V. M., & Barrow, P. A. (2003). Reduction of experimental *Salmonella* and *Campylobacter* contamination of chicken skin by application of lytic bacteriophages. *Applied and Environmental Microbiology, 69*(8), 5032–5036.

Guerin-Danan, C., Chabanet, C., Pedone, C., Popot, F., Vaissade, P., Bouley, C., et al. (1998). Milk fermented with yogurt cultures and *Lactobacillus casei* compared with yogurt and gelled milk: Influence on intestinal microflora in healthy infants. *The American Journal of Clinical Nutrition, 67*(1), 111–117.

Gul, P., & Bakht, J. (2015). Antimicrobial activity of turmeric extract and its potential use in food industry. *Journal of Food Science and Technology, 52*(4), 2272–2279.

Gutierrez, J., Barry-Ryan, C., & Bourke, P. (2008). The antimicrobial efficacy of plant essential oil combinations and interactions with food ingredients. *International Journal of Food Microbiology, 124*(1), 91–97.

Haddad, N., Burns, C. M., Bolla, J. M., Prévost, H., Fédérighi, M., & Drider, D. (2009). Long-term survival of *Campylobacter jejuni* at low temperatures is dependent on polynucleotide phosphorylase activity. *Applied and Environmental Microbiology, 75*(23), 7310–7318.

Hampton, T. (2013). Report reveals scope of US antibiotic resistance threat. *JAMA, 310*(16), 1661–1663.

Handy, R. D., & Shaw, B. J. (2007). Toxic effects of nanoparticles and nanomaterials: Implications for public health, risk assessment and the public perception of nanotechnology. *Health Risk Society, 9*(2), 125–144.

Hanning, I., Jarquin, R., & Slavik, M. (2008). *Campylobacter jejuni* as a secondary colonizer of poultry biofilms. *Journal of Applied Microbiology, 105*(4), 1199–1208.

Harborne, J. R. (1993). *Introduction to ecological biochemistry* (4th ed.). London: Elsevier.

Hermans, D., Martel, A., Van Deun, K., Van Immerseel, F., Heyndrickx, M., Haesebrouck, F., et al. (2011a). The cinnamon-oil ingredient trans-cinnamaldehyde fails to target *Campylobacter jejuni* strain KC 40 in the broiler chicken cecum despite marked *in vitro* activity. *Journal of Food Protection, 74*(10), 1729–1734.

Hermans, D., Pasmans, F., Messens, W., Martel, A., Van Immerseel, F., Rasschaert, G., et al. (2012). Poultry as a host for the zoonotic pathogen *Campylobacter jejuni*. *Vector-Borne and Zoonotic Diseases, 12*(2), 89–98.

Hermans, D., Van Deun, K., Martel, A., Van Immerseel, F., Messens, W., Heyndrickx, M., et al. (2011b). Colonization factors of *Campylobacter jejuni* in the chicken gut. *Veterinary Research, 42*(1), 82.

Higgins, J. P., Higgins, S. E., Guenther, K. L., Huff, W., Donoghue, A. M., Donoghue, D. J., et al. (2005). Use of a specific bacteriophage treatment to reduce *Salmonella* in poultry products. *Poultry Science, 84*(7), 1141–1145.

Hilbert, F., Scherwitzel, M., Paulsen, P., & Szostak, M. P. (2010). Survival of *Campylobacter jejuni* under conditions of atmospheric oxygen tension with the support of *Pseudomonas* spp. *Applied and Environmental Microbiology, 76*(17), 5911–5917.

Ho, K. (2001). Bacteriophage therapy for bacterial infections: Rekindling a memory from the pre-antibiotics era. *Perspectives in Biology and Medicine, 44*(1), 1–16.

Holzapfel, W. H., Geisen, R., & Schillinger, U. (1995). Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. *International Journal of Food Microbiology, 24*(3), 343–362.

Huff, W. E., Huff, G. R., Rath, N. C., Balog, J. M., & Donoghue, A. M. (2005). Alternatives to antibiotics: Utilization of bacteriophage to treat colibacillosis and prevent foodborne pathogens. *Poultry Science, 84*(4), 655–659.

Hyldgaard, M., Mygind, T., & Meyer, R. L. (2012). Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. *Frontiers in Microbiology, 3*, 12.

Isman, M. B. (2000). Plant essential oils for pest and disease management. *Crop Protection, 19*(8), 603–608.

Janež, N., & Loc-Carrillo, C. (2013). Use of phages to control Campylobacter spp. *Journal of Microbiological Methods, 95*(1), 68–75.

Joshua, G. P., Guthrie-Irons, C., Karlyshev, A. V., & Wren, B. W. (2006). Biofilm formation in Campylobacter jejuni. *Microbiology, 152*(2), 387–396.

Kaakoush, N. O., Castaño-Rodríguez, N., Mitchell, H. M., & Man, S. M. (2015). Global epidemiology of Campylobacter infection. *Clinical Microbiology Reviews, 28*(3), 687–720.

Kechagia, M., Basoulis, D., Konstantopoulou, S., Dimitriadi, D., Gyftopoulou, K., Skarmoutsou, N., et al. (2013). Health benefits of probiotics: A review. *ISRN Nutrition, 2013*, *7*.

Kennedy, D. O., & Wightman, E. L. (2011). Herbal extracts and phytochemicals: Plant secondary metabolites and the enhancement of human brain function. *Advances in Nutrition, 2*(1), 32–50.

Kittler, S., Fischer, S., Abdulmawjood, A., Glünder, G., & Klein, G. (2013). Effect of bacteriophage application on *Campylobacter jejuni* loads in commercial broiler flocks. *Applied and Environmental Microbiology, 79*(23), 7525–7533.

Koh, C. L., Sam, C. K., Yin, W. F., Tan, L. Y., Krishnan, T., Chong, Y. M., et al. (2013). Plant-derived natural products as sources of anti-quorum sensing compounds. *Sensors, 13*(5), 6217–6228.

Kollanoor Johny, A., Darre, M. J., Donoghue, A. M., Donoghue, D. J., & Venkitanarayanan, K. (2010). Antibacterial effect of trans-cinnamaldehyde, eugenol, carvacrol, and thymol on

Salmonella Enteritidis and *Campylobacter jejuni* in chicken cecal contents in vitro. *Journal of Applied Poultry Research, 19*(3), 237–244.

Kollanoor-Johny, A., Upadhyay, A., Baskaran, S. A., Upadhyaya, I., Mooyottu, S., Mishra, N., et al. (2012). Effect of therapeutic supplementation of the plant compounds trans-cinnamaldehyde and eugenol on *Salmonella enterica* serovar Enteritidis colonization in market-age broiler chickens 1 2. *Journal of Applied Poultry Research, 21*(4), 816–822.

Kovács, J. K., Felső, P., Makszin, L., Pápai, Z., Horváth, G., Ábrahám, H., et al. (2016). Antimicrobial and virulence-modulating effects of clove essential oil on the foodborne pathogen *Campylobacter jejuni*. *Applied and Environmental Microbiology, 82*(20), 6158–6166.

Kuroki, S., Saida, T., Nukina, M., Haruta, T., Yoshioka, M., Kobayashi, Y., et al. (1993). *Campylobacter jejuni* strains from patients with guillain-barré syndrome belong mostly to penner serogroup 19 and contain β-N-acetylglucosamine residues. *Annals of Neurology, 33*(3), 243–247.

Leriche, V., & Carpentier, B. (2000). Limitation of adhesion and growth of *Listeria monocytogenes* on stainless steel surfaces by *Staphylococcus sciuri* biofilms. *Journal of Applied Microbiology, 88*(4), 594–605.

Li, J., Dong, J., Qiu, J. Z., Wang, J. F., Luo, M. J., Li, H. E., et al. (2011). Peppermint oil decreases the production of virulence-associated exoproteins by *Staphylococcus aureus*. *Molecules, 16*(2), 1642–1654.

Lin, J. (2009). Novel approaches for *Campylobacter* control in poultry. *Foodborne Pathogens and Disease, 6*(7), 755–765.

Luangtongkum, T., Jeon, B., Han, J., Plummer, P., Logue, C. M., & Zhang, Q. (2009). Antibiotic resistance in Campylobacter: Emergence, transmission and persistence. *Future Microbiology, 4*(2), 189–200.

Magajna, B. A., & Schraft, H. (2015). *Campylobacter jejuni* biofilm cells become viable but non-culturable (VBNC) in low nutrient conditions at 4C more quickly than their planktonic counterparts. *Food Control, 50*, 45–50.

Mai, V., Ukhanova, M., Visone, L., Abuladze, T., & Sulakvelidze, A. (2010). Bacteriophage administration reduces the concentration of *Listeria monocytogenes* in the gastrointestinal tract and its translocation to spleen and liver in experimentally infected mice. *International Journal of Microbiology, 2010*, 624234.

Maura, D., Galtier, M., Le Bouguénec, C., & Debarbieux, L. (2012). Virulent bacteriophages can target O104: H4 enteroaggregative *Escherichia coli* in the mouse intestine. *Antimicrobial Agents and Chemotherapy, 56*(12), 6235–6242.

Maynard, A. D., Aitken, R. J., Butz, T., Colvin, V., Donaldson, K., Oberdörster, G., et al. (2006). Safe handling of nanotechnology. *Nature, 444*(7117), 267–269.

Merril, C. R., Scholl, D., & Adhya, S. L. (2003). The prospect for bacteriophage therapy in Western medicine. *Nature Reviews. Drug Discovery, 2*(6), 489.

Meunier, M., Guyard-Nicodème, M., Dory, D. and Chemaly, M., (2016). Control strategies against C ampylobacter at the poultry production level: biosecurity measures, feed additives and vaccination. *Journal of Applied Microbiology, 120*(5),1139–1173.

Moore-Neibel, K., Gerber, C., Patel, J., Friedman, M., & Ravishankar, S. (2012). Antimicrobial activity of lemongrass oil against *Salmonella enterica* on organic leafy greens. *Journal of Applied Microbiology, 112*(3), 485–492.

Morishita, T. Y., Aye, P. P., Harr, B. S., Cobb, C. W., & Clifford, J. R. (1997). Evaluation of an avian-specific probiotic to reduce the colonization and shedding of *Campylobacter jejuni* in broilers. *Avian Diseases, 41*(4), 850–855.

Murphy, C., Carroll, C., & Jordan, K. N. (2006). Environmental survival mechanisms of the foodborne pathogen *Campylobacter jejuni*. *Journal of Applied Microbiology, 100*(4), 623–632.

Nachamkin, I., Szymanski, C. M., & Blaser, M. J. (2008). *Campylobacter* (3rd ed.). Washington, DC: ASM Press.

National Research Council. (2007). *Toxicity testing in the 21st century: A vision and a strategy*. Washington, DC: National Academies Press.

Ncube, B., Finnie, J. F., & Van Staden, J. (2012). In vitro antimicrobial synergism within plant extract combinations from three South African medicinal bulbs. *Journal of Ethnopharmacology, 139*(1), 81–89.

Negi, P. S. (2012). Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. *International Journal of Food Microbiology, 156*(1), 7–17.

Nielsen, E. M., Engberg, J., Fussing, V., Petersen, L., Brogren, C. H., & On, S. L. (2000). Evaluation of phenotypic and genotypic methods for subtyping *Campylobacter jejuni* isolates from humans, poultry, and cattle. *Journal of Clinical Microbiology, 38*(10), 3800–3810.

Newell, D. G., Elvers, K. T., Dofper, D., Hansson, I., Jones, P., James, S., Gittins, J., Stern, N. J., Davies, R., Connerton, I. and Pearson, D. (2011). A critical review of biosecurity-based interventions and strategies to reduce Campylobacter on the poultry farm. *Applied and Environmental Microbiology*, AEM-01090.

Nikkhahi, F., Dallal, M. M. S., Alimohammadi, M., Foroushani, A. R., Rajabi, Z., Fardsanei, F., et al. (2017). Phage therapy: Assessment of the efficacy of a bacteriophage isolated in the treatment of salmonellosis induced by *Salmonella* Enteritidis in mice. *Gastroenterology and Hepatology from Bed to Bench, 10*(2), 131.

Nishiyama, K., Seto, Y., Yoshioka, K., Kakuda, T., Takai, S., Yamamoto, Y., et al. (2014). *Lactobacillus gasseri* SBT2055 reduces infection by and colonization of *Campylobacter jejuni*. *PLoS One, 9*(9), e108827.

Oakley, B. B., Lillehoj, H. S., Kogut, M. H., Kim, W. K., Maurer, J. J., Pedroso, A., et al. (2014). The chicken gastrointestinal microbiome. *FEMS Microbiology Letters, 360*(2), 100–112.

Oh, E., McMullen, L. M., Chui, L., & Jeon, B. (2017). Differential survival of hyper-aerotolerant *Campylobacter jejuni* under different gas conditions. *Frontiers in Microbiology, 8*, 954.

Olkkola, S., Culebro, A., Juntunen, P., Hänninen, M. L., & Rossi, M. (2016). Functional genomics in *Campylobacter coli* identified a novel streptomycin resistance gene located in a hypervariable genomic region. *Microbiology, 162*(7), 1157–1166.

Olszak, T., An, D., Zeissig, S., Vera, M. P., Richter, J., Franke, A., et al. (2012). Microbial exposure during early life has persistent effects on natural killer T cell function. *Science, 336*(6080), 489–493.

On, S. L. (2001). Taxonomy of *Campylobacter, Arcobacter, Helicobacter* and related bacteria: Current status, future prospects and immediate concerns. *Journal of Applied Microbiology, 90*(S6), 1S–15S.

Orquera, S., Gölz, G., Hertwig, S., Hammerl, J., Sparborth, D., Joldic, A., et al. (2012). Control of *Campylobacter* spp. and *Yersinia enterocolitica* by virulent bacteriophages. *Journal of Molecular and Genetic Medicine, 6*, 273.

Ouwehand, A. C., Derrien, M., de Vos, W., Tiihonen, K., & Rautonen, N. (2005). Prebiotics and other microbial substrates for gut functionality. *Current Opinion in Biotechnology, 16*(2), 212–217.

Parisi, C., Vigani, M., & Rodríguez-Cerezo, E. (2015). Agricultural nanotechnologies: What are the current possibilities? *Nano Today, 10*(2), 124–127.

Park, S. H., Lee, S. I., & Ricke, S. C. (2016). Microbial populations in naked neck chicken ceca raised on pasture flock fed with commercial yeast cell wall prebiotics via an Illumina MiSeq platform. *PLoS One, 11*(3), e0151944.

Parsaeimehr, M., Basti, A. A., Radmehr, B., Misaghi, A., Abbasifar, A., Karim, G., et al. (2010). Effect of *Zataria multiflora* boiss. Essential oil, nisin, and their combination on the production of enterotoxin C and α-hemolysin by *Staphylococcus aureus*. *Foodborne Pathogens and Disease, 7*(3), 299–305.

Persson, T., Hansen, T. H., Rasmussen, T. B., Skindersø, M. E., Givskov, M., & Nielsen, J. (2005). Rational design and synthesis of new quorum-sensing inhibitors derived from acylated homoserine lactones and natural products from garlic. *Organic & Biomolecular Chemistry, 3*(2), 253–262.

Pintar, K. D., Christidis, T., Thomas, M. K., Anderson, M., Nesbitt, A., Keithlin, J., et al. (2015). A systematic review and meta-analysis of the *Campylobacter* spp. prevalence and concentration

in household pets and petting zoo animals for use in exposure assessments. *PLoS One, 10*(12), e0144976.

Plummer, P., Sahin, O., Burrough, E., Sippy, R., Mou, K., Rabenold, J., et al. (2012). Critical role of LuxS in the virulence of *Campylobacter jejuni* in a guinea pig model of abortion. *Infection and Immunity, 80*(2), 585–593.

Qin, J., Li, R., Raes, J., Arumugam, M., Burgdorf, K. S., Manichanh, C., et al. (2010). A human gut microbial gene catalog established by metagenomic sequencing. *Nature, 464*(7285), 59.

Qiu, J., Wang, D., Xiang, H., Feng, H., Jiang, Y., Xia, L., et al. (2010). Subinhibitory concentrations of thymol reduce enterotoxins A and B and α-hemolysin production in *Staphylococcus aureus* isolates. *PLoS One, 5*(3), e9736.

Qiu, J., Wang, J., Luo, H., Du, X., Li, H., Luo, M., et al. (2011). The effects of subinhibitory concentrations of costus oil on virulence factor production in Staphylococcus aureus. *Journal of Applied Microbiology, 110*(1), 333–340.

Reeser, R. J., Medler, R. T., Billington, S. J., Jost, B. H., & Joens, L. A. (2007). Characterization of *Campylobacter jejuni* biofilms under defined growth conditions. *Applied and Environmental Microbiology, 73*(6), 1908–1913.

Reichling, J. (2010). Plant-Microbe Interactions and Secondary Metabolites with Antibacterial, Antifungal and Antiviral Properties. In *Annual plant reviews volume 39: Functions and biotechnology of plant secondary metabolites* (2nd ed., pp. 214–347). Oxford, UK: Blackwell.

Reuter, M., Mallett, A., Pearson, B. M., & van Vliet, A. H. (2010). Biofilm formation by Campylobacter jejuni is increased under aerobic conditions. *Applied and Environmental Microbiology, 76*(7), 2122–2128.

Ricke, S. C. (2015). Potential of fructooligosaccharide prebiotics in alternative and nonconventional poultry production systems 1. *Poultry Science, 94*(6), 1411–1418.

Rohwer, F. (2003). Global phage diversity. *Cell, 113*(2), 141.

Romero-Barrios, P., Hempen, M., Messens, W., Stella, P., & Hugas, M. (2013). Quantitative microbiological risk assessment (QMRA) of food-borne zoonoses at the European level. *Food Control, 29*(2), 343–349.

Rosenquist, H., Nielsen, N. L., Sommer, H. M., Nørrung, B., & Christensen, B. B. (2003). Quantitative risk assessment of human campylobacteriosis associated with thermophilic *Campylobacter* species in chickens. *International Journal of Food Microbiology, 83*(1), 87–103.

Saint-Cyr, M. J., Guyard-Nicodème, M., Messaoudi, S., Chemaly, M., Cappelier, J. M., Dousset, X., et al. (2016). Recent advances in screening of anti-Campylobacter activity in probiotics for use in poultry. *Frontiers in Microbiology, 7*, 553.

Sanders, M. E., & Marco, M. L. (2010). Food formats for effective delivery of probiotics. *Annual Review of Food Science and Technology, 1*, 65–85.

Sanders, S. Q., Boothe, D. H., Frank, J. F., & Arnold, J. W. (2007). Culture and detection of *Campylobacter jejuni* within mixed microbial populations of biofilms on stainless steel. *Journal of Food Protection, 70*(6), 1379–1385.

Santiesteban-López, A., Palou, E., & López-Malo, A. (2007). Susceptibility of food-borne bacteria to binary combinations of antimicrobials at selected aw and pH. *Journal of Applied Microbiology, 102*(2), 486–497.

Santini, C., Baffoni, L., Gaggia, F., Granata, M., Gasbarri, R., Di Gioia, D., et al. (2010). Characterization of probiotic strains: An application as feed additives in poultry against Campylobacter jejuni. *International Journal of Food Microbiology, 141*, S98–S108.

Sarker, S. A., McCallin, S., Barretto, C., Berger, B., Pittet, A. C., Sultana, S., et al. (2012). Oral T4-like phage cocktail application to healthy adult volunteers from Bangladesh. *Virology, 434*(2), 222–232.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., et al. (2011). Foodborne illness acquired in the United States—major pathogens. *Emerging Infectious Diseases, 17*(1), 7.

Scharff, R. L. (2012). Economic burden from health losses due to foodborne illness in the United States. *Journal of Food Protection, 75*(1), 123–131.

Schroeder, B. O., & Bäckhed, F. (2016). Signals from the gut microbiota to distant organs in physiology and disease. *Nature Medicine, 22*(10), 1079–1089.

Serban, D. E. (2014). Gastrointestinal cancers: Influence of gut microbiota, probiotics and prebiotics. *Cancer Letters, 345*, 258–270.

Sergeant, M. J., Constantinidou, C., Cogan, T. A., Bedford, M. R., Penn, C. W., & Pallen, M. J. (2014). Extensive microbial and functional diversity within the chicken cecal microbiome. *PLoS One, 9*(3), e91941.

Shin, H., Lee, J. H., Kim, H., Choi, Y., Heu, S., & Ryu, S. (2012). Receptor diversity and host interaction of bacteriophages infecting *Salmonella enterica* serovar Typhimurium. *PLoS One, 7*(8), e43392.

Shrestha, S., Donoghue, A., Arsi, K., Upadhyay, A., Wagle, B. R., Venkitanarayanan, K., et al. 2017. A carvacrol wash and/or a chitosan-based coating reduced *Campylobacter jejuni* on chicken wingettes. In *Poultry Science Association Annual Meeting*, Orlando, FL

Si, W., Gong, J., Chanas, C., Cui, S., Yu, H., Caballero, C., et al. (2006). In vitro assessment of antimicrobial activity of carvacrol, thymol and cinnamaldehyde towards Salmonella serotype Typhimurium DT104: Effects of pig diets and emulsification in hydrocolloids. *Journal of Applied Microbiology, 101*(6), 1282–1291.

Sillankorva, S. M., Oliveira, H., & Azeredo, J. (2012). Bacteriophages and their role in food safety. *International Journal of Microbiology, 2012*, 863945.

Sime-Ngando, T. (2014). Environmental bacteriophages: Viruses of microbes in aquatic ecosystems. *Frontiers in Microbiology, 5*, 355.

Simões, M., Simoes, L. C., & Vieira, M. J. (2010). A review of current and emergent biofilm control strategies. *LWT-Food Science and Technology, 43*(4), 573–583.

Siringan, P., Connerton, P. L., Payne, R. J., & Connerton, I. F. (2011). Bacteriophage-mediated dispersal of *Campylobacter jejuni* biofilms. *Applied and Environmental Microbiology, 77*(10), 3320–3326.

Skarp, C. P. A., Hänninen, M. L., & Rautelin, H. I. K. (2016). Campylobacteriosis: The role of poultry meat. *Clinical Microbiology and Infection, 22*(2), 103–109.

Smrekar, F., Ciringer, M., Peterka, M., Podgornik, A., & Štrancar, A. (2008). Purification and concentration of bacteriophage T4 using monolithic chromatographic supports. *Journal of Chromatography B, 861*(2), 177–180.

Sonnenburg, J. L., Xu, J., Leip, D. D., Chen, C. H., Westover, B. P., Weatherford, J., et al. (2005). Glycan foraging *in vivo* by an intestine-adapted bacterial symbiont. *Science, 307*(5717), 1955–1959.

Stern, N. J., Cox, N. A., Musgrove, M. T., & Park, C. M. (2001). Incidence and levels of *Campylobacter* in broilers after exposure to an inoculated seeder bird. *Journal of Applied Poultry Research, 10*(4), 315–318.

Sulakvelidze, A., & Barrow, P. (2005). Phage therapy in animals and agribusiness. In E. Kutter & A. Sulakvelidze (Eds.), *Bacteriophages: Biology and applications* (pp. 335–380). Boca Raton, FL: CRC Press.

Sulakvelidze, A. K., & Kutter, E. (2005). Bacteriophage therapy in humans. In *Bacteriophages: Biology and applications* (pp. 381–436). Boca Raton, FL: CRC Press.

Taboada, E. N., Clark, C. G., Sproston, E. L., & Carrillo, C. D. (2013). Current methods for molecular typing of *Campylobacter* species. *Journal of Microbiological Methods, 95*(1), 24–31.

Tang, Y., Sahin, O., Pavlovic, N., LeJeune, J., Carlson, J., Wu, Z., et al. (2017). Rising fluoroquinolone resistance in *Campylobacter* isolated from feedlot cattle in the United States. *Scientific Reports, 7*, 494.

Tareb, R., Bernardeau, M., Gueguen, M., & Vernoux, J. P. (2013). In vitro characterization of aggregation and adhesion properties of viable and heat-killed forms of two probiotic Lactobacillus strains and interaction with foodborne zoonotic bacteria, especially *Campylobacter jejuni*. *Journal of Medical Microbiology, 62*(4), 637–649.

Tassou, C. C., Drosinos, E. H., & Nychas, G. J. E. (1995). Effects of essential oil from mint (Mentha piperita) on Salmonella enteritidis and Listeria monocytogenes in model food systems at 4 and 10 C. *Journal of Applied Microbiology, 78*(6), 593–600.

Teh, K. H., Flint, S., & French, N. (2010). Biofilm formation by Campylobacter jejuni in controlled mixed-microbial populations. *International Journal of Food Microbiology, 143*(3), 118–124.

Thomrongsuwannakij, T., Chuanchuen, R., & Chansiripornchai, N. (2016). Identification of competitive exclusion and its ability to protect against *Campylobacter jejuni* in broilers. *The Thai Journal of Veterinary Medicine, 46*(2), 279–286.

Thongson, C., Davidson, P. M., Mahakarnchanakul, W., & Weiss, J. (2004). Antimicrobial activity of ultrasound-assisted solvent-extracted spices. *Letters in Applied Microbiology, 39*(5), 401–406.

Trachoo, N., & Frank, J. F. (2002). Effectiveness of chemical sanitizers against Campylobacter jejuni–containing biofilms. *Journal of Food Protection, 65*(7), 1117–1121.

Trigui, H., Paquet, V. E., Charette, S. J., & Faucher, S. P. (2016). Packaging of *Campylobacter jejuni* into multilamellar bodies by the ciliate *Tetrahymena pyriformis*. *Applied and Environmental Microbiology, 82*(9), 2783–2790.

Upadhyay, A., Arsi, K., Wagle, B. R., Upadhyaya, I., Shrestha, S., Donoghue, A. M., et al. (2017a). Trans-Cinnamaldehyde, Carvacrol, and Eugenol Reduce *Campylobacter jejuni* Colonization Factors and Expression of Virulence Genes in Vitro. *Frontiers in Microbiology, 8*, 713.

Upadhyay, A., Johny, A. K., Amalaradjou, M. A. R., Baskaran, S. A., Kim, K. S., & Venkitanarayanan, K. (2012). Plant-derived antimicrobials reduce *Listeria monocytogenes* virulence factors in vitro, and down-regulate expression of virulence genes. *International Journal of Food Microbiology, 157*(1), 88–94.

Upadhyay, A., Upadhyaya, I., Kollanoor-Johny, A., & Venkitanarayanan, K. (2013). Antibiofilm effect of plant derived antimicrobials on Listeria monocytogenes. *Food Microbiology, 36*(1), 79–89.

Upadhyay, A., Wagle, B. R., Shrestha, S., Upadhyaya, I., Arsi, K., Bhargava, K., et al. 2017b. Antimicrobial wash with Trans-cinnamaldehyde nanoemulsion reduces Campylobacter jejuni on chicken skin. In *Poultry Science Association Annual Meeting*, Orlando, FL.

Upadhyaya, I., Upadhyay, A., Kollanoor-Johny, A., Mooyottu, S., Baskaran, S. A., Yin, H. B., et al. (2015). In-feed supplementation of trans-cinnamaldehyde reduces layer-chicken egg-borne transmission of *Salmonella enterica* serovar Enteritidis. *Applied and Environmental Microbiology, 81*(9), 2985–2994.

Van Alphen, L. B., Burt, S. A., Veenendaal, A. K., Bleumink-Pluym, N. M., & Van Putten, J. P. (2012). The natural antimicrobial carvacrol inhibits *Campylobacter jejuni* motility and infection of epithelial cells. *PLoS One, 7*(9), e45343.

Venkitanarayanan, K., Kollanoor-Johny, A., Darre, M. J., Donoghue, A. M., & Donoghue, D. J. (2013). Use of plant-derived antimicrobials for improving the safety of poultry products. *Poultry Science, 92*(2), 493–501.

Wagenaar, J. A., French, N. P., & Havelaar, A. H. (2013). Preventing Campylobacter at the source: Why is it so difficult? *Clinical Infectious Diseases, 57*(11), 1600–1606.

Wagenaar, J. A., Newell, D. G., Kalupahana, R. S., & Mughini-Gras, L. (2015). Campylobacter: Animal reservoirs, human infections, and options for control. In *Zoonoses-Infections Affecting Humans and Animals* (pp. 159–177). Dordrecht, Netherlands: Springer.

Wagenaar, J. A., Van Bergen, M. A., Mueller, M. A., Wassenaar, T. M., & Carlton, R. M. (2005). Phage therapy reduces *Campylobacter jejuni* colonization in broilers. *Veterinary Microbiology, 109*(3), 275–283.

Wagle, B. R., Donoghue, A. M., Arsi, K., Upadhyay, A., Shrestha, S., Blore, P.J., et al. (2016). Eugenol wash and chitosan-based coating reduces *Campylobacter jejuni* counts on poultry products. In *Poultry Science Association Annual Meeting*, New Orleans, LA.

Wagle, B. R., Upadhyay, A., Arsi, K., Shrestha, S., Venkitanarayanan, K., Donoghue, A. M., et al. (2017a). Application of β-resorcylic acid as potential antimicrobial feed additive to reduce *Campylobacter* colonization in broiler chickens. *Frontiers in Microbiology, 8*, 599.

Wagle, B. R., Upadhyay, A., Arsi, K., Upadhyaya, I., Shrestha, S., Blore, P.J., et al. (2017b). Phytochemicals reduce biofilm formation and inactivates mature biofilm of *Campylobacter jejuni.* In *Poultry Science Association Annual Meeting*, Orlando, FL.

Wagner, R. D., Johnson, S. J., & Kurniasih Rubin, D. (2009). Probiotic bacteria are antagonistic to *Salmonella enterica* and *Campylobacter jejuni* and influence host lymphocyte responses in human microbiota-associated immunodeficient and immunocompetent mice. *Molecular Nutrition & Food Research, 53*(3), 377–388.

Wassenaar, T. M., & Newell, D. G. (2000). Genotyping of Campylobacter spp. *Applied and Environmental Microbiology, 66*(1), 1–9.

Weber-Dąbrowska, B., Mulczyk, M., & Górski, A. (2001). Bacteriophage therapy of bacterial infections: An update of our institute's experience. In *Inflammation* (pp. 201–209). Dordrecht, Netherlands: Springer.

Wine, E., Gareau, M. G., Johnson-Henry, K., & Sherman, P. M. (2009). Strain-specific probiotic (*Lactobacillus helveticus*) inhibition of *Campylobacter jejuni* invasion of human intestinal epithelial cells. *FEMS Microbiology Letters, 300*(1), 146–152.

Woo-Ming, A., Arsi, K., Donoghue, A. M., Moyle, J. R., & Donoghue, D. J. (2016). Efficacy of natural cranberry extracts against *Campylobacter* colonization in poultry. *International Journal of Food Engineering, 2*, 66–70.

Yin, H. B., Chen, C. H., Kollanoor-Johny, A., Darre, M. J., & Venkitanarayanan, K. (2015). Controlling *Aspergillus flavus* and *Aspergillus parasiticus* growth and aflatoxin production in poultry feed using carvacrol and trans-cinnamaldehyde. *Poultry Science, 94*(9), 2183–2190.

Young, K. T., Davis, L. M., & DiRita, V. J. (2007). *Campylobacter jejuni*: Molecular biology and pathogenesis. *Nature Reviews. Microbiology, 5*(9), 665.

Zhao, T., Doyle, M. P., & Zhao, P. (2004). Control of *Listeria monocytogenes* in a biofilm by competitive-exclusion microorganisms. *Applied and Environmental Microbiology, 70*(7), 3996–4003.

Chapter 5
Pre-harvest Approaches to Improve Poultry Meat Safety

Mary Anne Amalaradjou

5.1 Introduction

Salmonella and *Campylobacter* infections are a major cause of gastroenteritis in humans and are of significant public health concern in the United States and worldwide (Alali and Hofacre 2016, Vandeplas et al. 2010). In the United States, it is estimated that approximately 1 million and 0.8 million cases of foodborne salmonellosis and campylobacteriosis, respectively, occur every year (Scallan et al. 2011). Although other food sources have been implicated in these outbreaks, these infections are often associated with the consumption of raw or undercooked poultry (Rajan et al. 2017, Sahin et al. 2015). It is estimated that 10–29% of salmonellosis and 43% of campylobacteriosis are associated with poultry products (Painter et al. 2013). This link between zoonotic foodborne infection and poultry meat is concerning given the tremendous increase in the demand for and production of poultry meat. Approximately 37.8 billion pounds of broiler meat was produced in the United States in 2013 (Mathews 2014). Further, poultry meat constitutes approximately 50% of the annual per capita consumption of meat in the United States (Rajan et al. 2017). With increasing poultry meat consumption, the safety of poultry products is a priority to consumers, producers, and the US government (Alali and Hofacre 2016).

Several factors influence *Salmonella* and *Campylobacter* colonization in broilers including genetics, age, stress due to overcrowding or illness, level of pathogen exposure, virulence of the pathogen, and dysbiosis (Bailey 1988, Cox et al. 2000, Lee and Newell 2006). Broilers are most susceptible to pathogen colonization immediately after and a few days post-hatch via vertical transmission (in ovo) or horizontal transmission at the hatchery, during transportation or on the grow-out

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farms (Alali and Hofacre 2016, Bailey 1988, Lahellec and Colin 1985). At the broiler farms, stress associated with feed withdrawal prior to slaughter, contaminated litter, feed and water, and alterations in the intestinal microbiome can predispose the birds to *Salmonella* and *Campylobacter* colonization (Byrd et al. 1998, Ramirez et al. 1997). Due to the varied factors that promote enteropathogen presence, colonization, and shedding in chicken, control strategies to reduce these zoonotic bacteria need to be multitiered and goal-oriented providing flexibility to producers and processors (Umaraw et al. 2017).

Pre-harvest, post-harvest, and retail are the primary areas for researchers and policy makers to target in reducing foodborne pathogens in poultry meat and meat products. Each of these areas is unique in terms of the challenges associated with understanding the source and transmission of the pathogen, risk factors, critical control points, and intervention strategies. With reference to policy and regulations, the post-harvest environment has been at the forefront of the discussion due to its proximity to the finished product and consumer risk. Further, the processing environment is more streamlined and controlled as opposed to the open and complex nature of the pre-harvest production system (Alali and Hofacre 2016, Doyle and Erickson 2012, Torrence 2016). Nevertheless, the primary production environment is the start of the food production line, and therefore implementation of intervention strategies at the pre-harvest stage would help control the entry and horizontal transmission of pathogens along the food production continuum.

On the farm, the contamination cycle begins with the infection of the animal and proceeds by shedding of pathogens in the feces, which in turn contaminates the environment and leads to new infections or reinfection of animals (Oliver et al. 2009, Rajan et al. 2017, Sahin et al. 2015). In fact, pathogen amplification in the animal, fecal shedding, and environmental contamination are responsible for pathogen persistence on the farm (Berry and Wells 2016). Given this cycle of contamination and recontamination on the farm premises, complete elimination of the pathogen may be unlikely in the pre-harvest environment. Nevertheless, measures aimed at reducing infection levels, number, and persistence of pathogens will have a positive impact on decreasing foodborne contamination farther down the food production chain and ultimately reduce public health risks. This chapter discusses the different interventions that can be applied toward the pre-harvest control of *Salmonella* and *Campylobacter* in broiler chickens.

5.2 Pre-harvest Interventions

The central theme surrounding pre-harvest food safety includes the food animal, its microbial ecology, and the interaction of the animal with its surrounding environment (Torrence 2016). On the poultry farm, pathogens can be introduced or transmitted through a wide variety of carriers or fomites including water, litter, air,

vehicles, equipment, workers, wildlife, birds, insects, and rodents (Rajan et al. 2017, Sahin et al. 2015). Therefore, unlike the meat processing environment, developing and implementing mitigation strategies is more challenging on the production site. The foremost approach undertaken by the poultry industry to reduce environmental exposure and transmission is via the implementation of good animal management practices consisting of hygiene, disinfection, and biosecurity measures at the breeder house, hatchery, and grow-out farms (Berry and Wells 2016, Cox and Pavic 2010, Doyle and Erickson 2012, Umaraw et al. 2017). Interventions within the animal have been an important focus of the research community in promoting the food safety of poultry meat and meat products. In this regard, general strategies that have been employed in the pre-harvest control of *Salmonella* and *Campylobacter* in broiler chicken include (1) preventing the introduction of infections and reducing environmental exposure (biosecurity measures), (2) preventing the survival and spread of the infection within the flock (antimicrobial interventions, nutritional strategies), and (3) increasing host resistance to reduce pathogen carriage in the gut (competitive exclusion, vaccination, and host genetics selection, Berry and Wells 2016, Umaraw et al. 2017, Lin, 2009). Figure 5.1 summarizes the different pre-harvest intervention strategies that are employed to control and mitigate *Salmonella* and *Campylobacter* in broiler production.

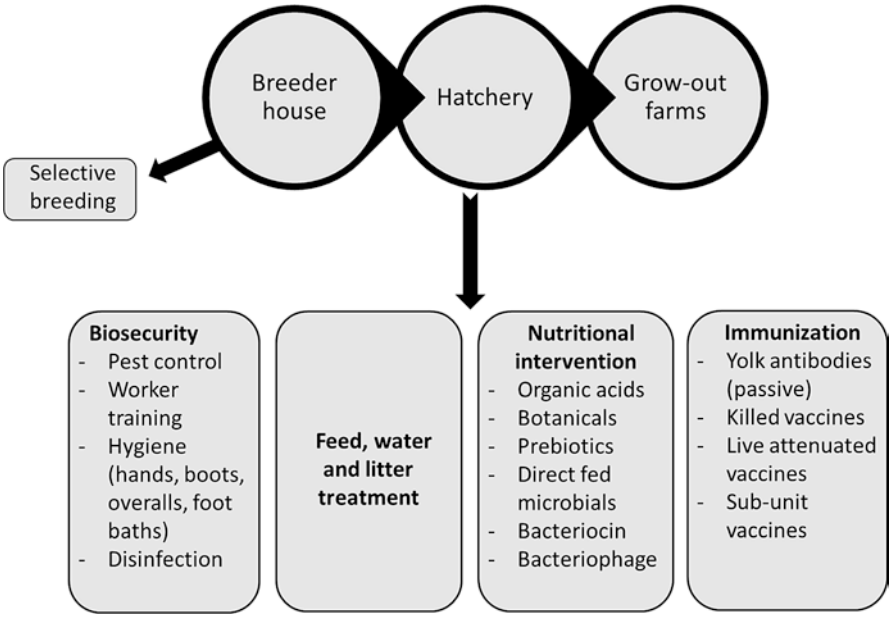

Fig. 5.1 Pre-harvest control of *Salmonella* and *Campylobacter* in broiler chicken

5.2.1 Biosecurity and Hygienic Measures

Hygiene, disinfection, and biosecurity measures are at the heart of an effective pre-harvest food safety program (Berry and Wells 2016). Biosecurity measures encompass action plans designed to prevent and control pathogens from entering the breeder, hatchery, and broiler houses. This is accomplished by preventing and controlling wildlife, rodents, insects, unauthorized human visitors, and fomites (feed, truck, farm equipment) since they can all act as potential vectors or carriers of *Salmonella* and *Campylobacter* (Alali and Hofacre 2016, Bailey et al. 2001, Marin et al. 2011, Roche et al. 2009, Skov et al. 2008, Stern et al. 2001). Several measures that can be adopted on the farm as part of their biosecurity measures include (1) design of poultry houses to prevent entry of vectors; (2) use of pest control measures such as traps and baits; (3) regular monitoring and prompt disposal of trapped vectors; (4) limiting access to the flocks to only essential personnel; (5) training workers in best hygienic practices; (6) provision of footbaths, hand washing stations, disposable coveralls, and overshoes; (7) minimizing or elimination of shared equipment and transportation vehicles with other farms; and (8) effective decontamination of equipment and vehicles before entry on the farm (Alali and Hofacre 2016, Sahin et al. 2015).

Research-based evidence has demonstrated that incorporation of these approaches has reduced pathogen prevalence on poultry farms (Evans and Sayers 2000, Humphrey et al. 1993, Le Bouquin et al. 2010, Marin et al. 2011). For example, Allain et al. (2014) demonstrated that rodent control around broiler houses was associated with a lower risk of *Campylobacter* colonization. Additionally, Marin et al. (2011) and Wedderkopp et al. (2001) observed that hygienic practices including workers washing hands with antiseptic soap prior to entering the broiler house, regularly checking on rodent baits, proper disposal of dead birds, use of combined surface and pulse-fogging disinfection methods, and cleaning and disinfection prior to placement of new flock significantly reduced the risk for *Salmonella* contamination in broilers. Furthermore, practice of using footbaths prior to entering the broiler house and frequency of changing the dipping solution was found to reduce *Campylobacter* colonization in birds (Evans and Sayers 2000, Humphrey et al. 1993).

Besides the above mentioned sources, litter, feed, and water can also serve as fomites in the transmission of *Salmonella* and *Campylobacter* (Bailey et al. 2001, Doyle and Erickson 2012, Gregory et al. 1997). In this regard, water sanitation and feed treatment are important to prevent horizontal dissemination of the pathogen (Johny et al. 2008, Line and Bailey 2006, Maciorowski et al. 2004, Meunier et al. 2016, Umaraw et al. 2017). The addition of organic acids such as lactic acid and chlorination can be used to control enteric pathogens in water (Boxall et al. 2003, Chaveerach et al. 2002). In the case of feed, treatments including heat, addition of acids, or formaldehyde have been shown to be effective in controlling *Salmonella* and *Campylobacter* populations (Jones 2011, Umaraw et al. 2017). Additional treatments that can be applied to feed and water to reduce pathogen populations have been discussed under nutritional interventions. Litter treatments to control

Salmonella and *Campylobacter* are discussed under a Sect. 5.2.4. Overall these findings indicate that proper application of biosecurity measures can reduce pathogen introduction into and contamination on the farm. However, since the bird is under constant risk for contamination, such hygiene practices are only partially effective. Thus complementary measures are necessary to efficiently control *Salmonella* and *Campylobacter* at the primary production site (Hermans et al. 2011, Vandeplas et al. 2010).

5.2.2 Nutritional Strategies

These strategies are applied on the farm at the production level to reduce pathogen load and prevalence in live birds. The antimicrobial products are primarily administered to poultry through supplementation in feed and water.

5.2.2.1 Organic Acids

Organic acids including short (SCFA; formic, acetic, propionic, and butyric acid)- and medium-chain fatty acids (MCFA; caprylic, capric, caproic, and lauric acid) have been demonstrated to exert bacteriostatic or bactericidal effects against Gram-negative bacteria including *Salmonella* and *Campylobacter* in vitro and in vivo (Ricke 2003, Thompson and Hinton 1997, Van Immerseel et al. 2006, Vandeplas et al. 2010). Supplementation of organic acids to chicken serves two purposes, namely, (1) to control pathogen load in feed and water and (2) to reduce pathogen colonization and shedding in the birds (Meunier et al. 2016, Vandeplas et al. 2010, Wales et al. 2013). These acids act by reducing intracellular pH in the pathogen thereby inhibiting several key physiological functions within the bacterial cell eventually leading to cell death (Durant et al. 2000, Thompson and Hinton 1997, Vandeplas et al. 2010). Furthermore, the acids are also known to inhibit key virulence determinants in *Salmonella* including *hilA*, *invF*, *sipC*, and *hilD* (Durant et al. 2000, Gantois et al. 2006). The organic acids are usually incorporated at a 0.5–3% inclusion rate depending on acceptability to birds, toxicity, and buffering capacity of the feed and acid (Berge and Wierup 2012, Ricke 2003). With reference to the buffering capacity, it is found to be highest in minerals followed by protein feedstuff and lowest in cereal and cereal by-products (Berge and Wierup 2012).

Although the initial application of acid was intended toward feed and carcass decontamination, the observation that feeding acid-treated feed (formic and propionic acid) to chickens reduced cecal *Salmonella* populations (*S*. Enteritidis, *S*. Typhimurium, *S*. Agona) by greater than 7 $\log_{10}$ relative to control (9 $\log_{10}$) clearly established the ability of acid to control colonization and shedding in the birds (Iba and Berchieri 1995). Several studies have been performed with the aim of specifically controlling *Salmonella* and *Campylobacter* populations in birds. Hinton et al. (1985) demonstrated that in-feed supplementation of formic and propionic acid

reduced shedding and cecal colonization of *Salmonella* in naturally infected birds. In fact, 50% of all untreated birds had *Salmonella*-positive cloacal and cecal contents, while none were detected in the birds that consumed acid-treated feed. Additionally, a 3-year cumulative study reported a reduction in numbers of *Salmonella*-positive hatchlings when breeder stocks were given formic acid-treated feed (Humphrey and Lanning 1988). Similar antimicrobial efficacy was observed when the acids were supplemented through drinking water. Supplementation of water containing acetic, lactic, or formic acid (0.5%) reduced crop contamination following *S*. Typhimurium challenge during the feed withdrawal period (Byrd et al. 2001).

When compared to SCFA, MCFA have been found to be more effective in reducing *Salmonella* populations in live birds. Van Immerseel et al. (2004) demonstrated that prophylactic administration of caproic acid (3 g/kg of feed) significantly reduced *S*. Enteritidis colonization of ceca and internal organs in week-old chicks. Additionally they observed that this reduced pathogen colonization was associated with a corresponding attenuation in virulence gene expression in *Salmonella.* Similar to caproic acid, in-feed administration of caprylic acid (0.7% and 1%) reduced *S*. Enteritidis populations in the ceca (2.5 $\log_{10}$) and liver (2 $\log_{10}$) of caprylic acid-fed 10-day-old birds when compared to the corresponding control birds (Johny et al. 2009). Besides prophylaxis, caprylic acid was also investigated for its therapeutic ability to control *Salmonella* in broilers. Broiler chicks were infected with *Salmonella* by crop gavage on day 25 and maintained until 6 weeks of age. A subset of *Salmonella*-infected birds were provided with caprylic acid containing feed during the last 5 days of the study following which ceca were sampled for *Salmonella* population. Results of this study revealed that caprylic acid was effective in reducing pathogen populations in the ceca, small intestine, cloaca, liver, and spleen of 6-week-old birds (Kollanoor-Johny et al. 2012).

As with *Salmonella*, supplementation of acids has proven to be effective in controlling *Campylobacter jejuni* in chicken. Addition of formic acid (1.5–2%) in combination with sorbate (0.1%) reduced cecal *Campylobacter* populations to below detection limits in comparison to control birds (6 $\log_{10}$; Skanseng et al. 2010). In addition to formic acid, the use of caprylic, capric, caproic, and butyric acid has been found to be effective in controlling *Campylobacter jejuni*. In-feed supplementation of caprylic acid (0.7–0.875%) was found to significantly reduce cecal colonization of *Campylobacter* in 10-day-old birds (Solis de Los Santos et al. 2008a). Similarly, therapeutic supplementation of caprylic acid (0.7% and 1.4%) reduced pathogen population by 3–4 logs compared to the control (Solis de Los Santos et al. 2008b). Although use of organic acids has met with some success, a meta-analysis study by Totton and others Totton et al. (2012) found the effectiveness of these acids to be inconsistent. This variability in efficacy can be attributed to product differences, dosage, mode of application, infection protocol, lengths of follow-up, quality of studies, and measurement of the outcome (Vandeplas et al. 2010). In spite of these limitations, SCFAs and MCFAs can be an effective arsenal that the farmer could use to reduce pathogen carriage and infection pressure on the farm.

5.2.2.2 Botanicals

Botanicals or phytochemicals are made up of primary and secondary components. The secondary products are also described as essential oils, colorants, and phenolics (Diaz-Sanchez et al. 2015). Historically, plant extracts and essential oils have been used to preserve foods as well as to enhance food flavor. Further, these compounds have been widely used as prophylactics and therapeutics in promoting and maintaining human health. These secondary plant compounds do not play a direct role in plant physiology but are critical to enhancing plant fitness and protection from diseases (Upadhyay et al. 2014). In effect most of these metabolites are produced as a result of reciprocal interactions between plants, microorganisms, and animals (Reichling et al. 2009, Upadhyay et al. 2014). Over the years, approximately 3000 essential oils have been identified, and almost 300 of these are commercially used in pharmaceutical, agronomical, food, and cosmetic industries as alternatives to synthetic chemicals (Bakkali et al. 2008, Diaz-Sanchez et al. 2015).

In the last decade, identification of the potent antimicrobial properties of these phytochemicals has led to a renewed interest for their application in the food industry and animal agriculture. The antimicrobial property of these essential oils can be attributed to their constituents including terpenoids, phenolics, glycosides, alkaloids, flavonoids, and glucosinolates (Upadhyay et al. 2014, Wenk 2006). These molecules exert their antimicrobial activity via multiple mechanisms including (1) disruption of bacterial cellular membrane, (2) modification of bacterial surface hydrophobicity, (3) stimulating immune response in the host, (4) inhibiting pathogen colonization, (5) attenuating pathogen virulence, and (6) modulating the host microbiome by promoting growth of beneficial bacteria including *Lactobacilli* and *Bifidobacteria* (Diaz-Sanchez et al. 2015, Upadhyay et al. 2014, Vidanarachchi et al. 2005, Windisch et al. 2008). In addition to their varied mechanism of action, there are several advantages associated with the use of phytobiotics. Compared with antibiotics, synthetic chemicals, and inorganics, essential oils are natural, less toxic, and residue-free (Upadhyay et al. 2014). In addition, many are certified as generally recognized as safe (GRAS) by the FDA and therefore make them ideal candidates for use in conventional and organic agricultural and food systems (Darre et al. 2014, Wang et al. 1998).

The antimicrobial properties of several plant-derived antimicrobials against *Salmonella* and *Campylobacter* have been previously reported in vitro and in vivo (Vandeplas et al. 2010, Hermans et al. 2011, Kurekci et al. 2014, Kollanoor-Johny et al. 2012, Johny et al. 2008, Arsi et al. 2014, Upadhyaya et al. 2015). Kollanoor Johny et al. (2010) demonstrated the antimicrobial properties of trans-cinnamaldehyde, carvacrol, and eugenol against *Salmonella* and *Campylobacter* in chicken cecal contents in vitro. They observed that trans-cinnamaldehyde (25 mM) completely inactivated *Salmonella* and reduced *Campylobacter* load by less than 1 $\log_{10}$ by 8 h of incubation. Similarly, trans-cinnamaldehyde was found to be effective in inactivating *Salmonella* and *Campylobacter* in chicken drinking water when tested at low and high ambient temperatures (Johny et al. 2008).

Beyond in vitro trials, in-feed supplementation of select phytochemicals was found to be effective in reducing pathogen populations in chickens. Prophylactic supplementation of cinnamaldehyde (1%) and eugenol (0.75%) significantly reduced cecal *Salmonella* populations by greater than 3 $\log_{10}$ when compared to the control (Kollanoor-Johny et al. 2012). Similarly incorporation of thymol (0.25%), carvacrol (1%), thymol (2%), and a combination of thymol and carvacrol at 0.5% was found to reduce *Campylobacter* populations in the ceca of broiler chickens (Arsi et al. 2014). Besides these phytochemicals, capsaicin (18 ppm), capsaicin (5 ppm), and oleoresin (20 ppm) have been shown to be effective in reducing *Salmonella* colonization in the ceca (Orndorff et al. 2005, Tellez et al. 1993, Vicente et al. 2007). Additionally administration of thymol-β-D-glucopyranoside decreased *Campylobacter jejuni* levels in the chicken crop (Epps et al. 2015). Although these studies demonstrate a potential for the use of essential oils in reducing *Salmonella* and *Campylobacter* in poultry, several questions need to be answered before they can be commercially applied. These include identification of ideal dosage that does not impact animal growth, feed intake and productivity, miscibility and compatibility with the feed ingredients, commercial availability in quantities required by the poultry industry, and economic feasibility (Darre et al. 2014).

5.2.2.3 Prebiotics

Prebiotics consist of nondigestible carbohydrates that are minimally metabolized by the host as they pass through the upper GIT and are available for use by the intestinal flora. In the lower gut, prebiotics selectively stimulate the growth and activity of one or more bacteria that are beneficial to the host (Alali and Hofacre 2016, Vandeplas et al. 2010). Prebiotic application in food animals is based on their ability to inhibit intestinal colonization by enteric pathogens (Grizard and Barthomeuf 1999, Rehman et al. 2009). This prebiotic-mediated colonization resistance could be due to direct binding of these carbohydrates with the pathogen and therefore blocking adhesion of these bacteria to host cells (Spring et al. 2000). Indirectly, by serving as fermentable substrates to the commensal bacteria and promoting their growth and metabolism, prebiotics could exclude pathogens by competitive exclusion. Additionally, prebiotic supplementation enhances production of volatile fatty acids including lactic acid and bacteriocins which are inhibitory to pathogens (Šušković et al. 2001).

Over the last several years, researchers have investigated the application of prebiotics in poultry production and assessed their effect on gut health, performance, and reduction of pathogen shedding (Gaggia et al. 2010, Yang et al. 2007). Fructose oligosaccharides (FOS) and mannanoligosaccharides (MOS) are the most extensively studied oligosaccharides in chicken with reference to their prebiotic potential and anti-*Salmonella* activity (Bailey et al. 1991, Choi et al. 1994, Ricke 2015, Spring et al. 2000). Supplementation of MOS (4000 ppm) to chicks (3-day-old) artificially inoculated with *S*. Typhimurium (4 log CFU/bird) showed a tenfold reduction in cecal pathogen populations at the end of study (Spring et al. 2000). In another

series of experiments, birds challenged with *S*. Dublin were provided with MOS which significantly reduced the number of birds positive for *Salmonella* in comparison to the control (56% versus 90%, Spring et al. 2000). Further, Fernandez et al. (2000) studied the protection against *S*. Enteritidis cecal colonization in birds that were inoculated with cecal contents from hens fed a MOS-supplemented diet. They observed that birds fed on a diet containing MOS and inoculated with cecal culture from MOS-treated hens were better protected from *Salmonella* colonization. A reduction in the number of *Salmonella*-positive birds was also observed in the MOS-fed group (Fernandez et al. 2002). Similar reduction in *Campylobacter* colonization was seen in MOS-fed chickens that were infected with ten different strains of *C. jejuni* and *C. coli* (Anderson et al. 2005).

With regard to FOS, dietary supplementation studies in poultry have yielded mixed results (Jacob and Pescatore 2012, Ricke 2015). Feeding FOS (7.5 g/kg of feed) to young chicks challenged with *S*. Typhimurium had little influence on the cecal colonization of *Salmonella* (Bailey et al. 1991). However, feeding FOS to birds stressed by feed and water withdrawal resulted in a reduction in cecal S. Typhimurium load (Bailey et al. 1991). A similar change in *Salmonella* colonization was observed in stressed birds after 18 days of FOS feeding (Choi et al. 1994). It was hypothesized that the observed antimicrobial effect could be due to a shift in intestinal microbiome throughout the FOS-feeding period (Bailey et al. 1991). Although FOS by itself exerted minimal effect against *Salmonella*, Yusrizal and Chen (2003) observed a reduction in *Campylobacter* levels in the large intestine of 42-day-old birds fed chicory fructans. Thitaram et al. (2005) investigated the prebiotic effect of isomalto-oligosaccharide (IMO) in reducing pathogen colonization. Feeding IMO (1%) reduced cecal *S*. Typhimurium populations when compared to the control.

Besides these oligosaccharides, lactose supplementation has shown some promising results. Dietary supplementation of lactose through feed or water was shown to decrease *Salmonella* colonization in broilers (Barnhart et al. 1999, Corrier et al. 1990, Hinton et al. 1990, 1991, Tellez et al. 1993). Although not a prebiotic in the ideal sense, it is believed that lactose promotes the growth of lactose-fermenting bacteria that either outcompete *Salmonella* or produce substances toxic to the pathogen (DeLoach et al. 1990, Rehman et al. 2009). As evident from these studies, the use of prebiotics to control pathogens in chicken has been associated with inconsistent results. Since not all commensals can utilize the prebiotic, changes in their abundance would not necessarily translate into increased pathogen reduction. In addition to the chemical nature of the prebiotic, purity, source, and dosage could also account for the heterogeneity observed in results with prebiotics.

5.2.2.4 Direct-Fed Microbials

Direct-fed microbials (DFM) consist of live organisms and spores that can be fed to poultry either to promote their performance and/or control pathogens (Patterson and Burkholder 2003). From a food safety aspect, these organisms can include defined and characterized cultures (probiotics) or mixed partially or unidentified cultures

(competitive exclusion flora—CE) that have demonstrated efficacy against poultry pathogens (Doyle and Erickson 2012). The concept of CE or "Nurmi concept" came about following the research by Rantala and Nurmi (1973) who demonstrated that administration of normal intestinal microbiota from healthy adult birds to chicks protected the young birds from invading pathogens. Since this initial finding, several studies have been performed to identify effective CE that could be applied in the control of *Salmonella* and *Campylobacter* in chicken. CE application is primarily targeted for newly hatched chicks or turkey poults that are highly susceptible to *Salmonella* and *Campylobacter* colonization due to their underdeveloped gut microbiome (Alali and Hofacre 2016). It is expected that transfer of microbiota from healthy adult birds will help populate the naive intestinal tract of these young birds and thereby exclude pathogen colonization (Nisbet 2002, Zhang et al. 2007a, b). Although initially applied in the first drinking water to hatchlings, spraying CE cultures either at the hatchery or on the grow-out farms has been shown to be more effective (Goren et al. 1988, Goren et al. 1984, Pivnick and Nurmi 1982). Further, Blankenship et al. (1993) demonstrated that initial spraying in the hatchery followed by CE administration in drinking water was effective in controlling *Salmonella* infection in poultry.

The ability of undefined cultures to reduce pathogen populations led to the development of several commercial products such as Aviguard®, FM-B11®, Primalac®, MSC®, and Avifree® (Carter et al. 2009). All of these products have demonstrated varying degrees of efficacy in reducing *Salmonella* and *Campylobacter* colonization in chicken. For example, following in-feed supplementation with Primalac® (100 mg/kg), Gharib Naseri et al. (2012) observed cecal and fecal reduction in *Campylobacter* populations by 0.9 and 0.8 log CFU, respectively. Similarly, oral supplementation of Primalac® resulted in a 1 log CFU/g reduction in *Salmonella* counts in turkey poults (Grimes et al. 2008). One of the important challenges with the use of CE is the verification of its bacterial compositions. Commercially available cultures for competitive exclusion are usually made of mixed cecal cultures. These mixed cultures may contain opportunistic bacteria that could be pathogenic to poultry and also lead to variability in their efficacy (Yamazaki et al. 2012). Kerr et al. (2013) reviewed the efficacies of various commercial CE products for reducing *Salmonella* colonization and observed that undefined CE products outperformed the commercial products. However, its practical application is plagued by the lack of proof of consistent efficacy. Although CE products are commercially available, due to concerns regarding the potential for virulence and transfer of antibiotic resistance genes from undefined bacteria, their use in food animals is not approved in the United States (Doyle and Erickson 2012).

An alternative approach to the use of undefined or partially defined cecal cultures could be probiotics carefully selected and characterized for their CE potential (Mountzouris et al. 2009). The use of probiotics has been suggested as an effective strategy to reduce *Salmonella* and *Campylobacter* infection in chicken (Gusils et al. 1999, Van Coillie et al. 2007). Most commonly employed probiotics in food animals include members of the genera *Lactobacillus*, *Enterococcus*, *Bacillus*, and *Saccharomyces* (Saint-Cyr et al. 2016, Vandeplas et al. 2010). In this regard,

Van Coillie et al. (2007) observed that inoculation of *L. reuteri* R-17485 and *L. johnsonii* into the proventriculus significantly reduced *S*. Enteritidis colonization in the cecum, liver, and spleen of 6-day-old chicks. Inoculation with *Enterococcus* spp. was also found to produce similar results (Audisio et al. 2000). Besides inoculations, Line et al. (1998) found that sustained in-feed supplementation of *Saccharomyces boulardii* reduced cecal contamination from 1.64 to 0.15 log CFU/g in broilers challenged with *S*. Typhimurium and *C. jejuni*. Recently, Cean et al. (2015) observed approximately an 8 log and 1 log reduction in duodenal and cecal colonization of *C. jejuni* in birds fed *L. paracasei* J. R, *L. rhamnosus* 15b, *Lactococcus lactis* Y, and *L. lactis* FOa.

Beyond using probiotics, probiotic cocktails, and prebiotics, researchers have looked into the application of probiotics in conjunction with prebiotics (synbiotics) as a means to promote the anti-pathogenic effect of the individual components (Collins and Gibson 1999, Schrezenmeir and de Vrese 2001, Vandeplas et al. 2010). Nisbet et al. (1993) employed a combination of lactose and CE flora to demonstrate a higher reduction in *Salmonella* colonization in chicken compared to lactose or CE alone. Similarly, supplementation of CE with 0.04% MOS reduced *Campylobacter* loads in the cecum by greater than 3 log CFU/g in comparison to CE or MOS alone (Arsi et al. 2015). Concerning synbiotic use in poultry, most studies have used prebiotics in combination with CE. However, research is currently being pursued on the use of defined cultures including probiotics and prebiotics with identified anti-*Salmonella*/*Campylobacter* properties. Baffoni et al. (2012) evaluated the therapeutic potential of *Bifidobacterium longum* PCB133 and 3% galactooligosaccharide to reduce *C. jejuni* colonization in broiler chicks. The in vivo study demonstrated a significantly reduced *C. jejuni* concentration in poultry feces in chickens administered the synbiotic relative to other treatment groups. Further, sustained administration of *B. longum* in combination with xylooligosaccharide was found to reduce cecal *Campylobacter* populations in 14-day-old birds (Baffoni et al. 2017). Given the potential of this antimicrobial approach, identification of effective probiotics or synbiotic treatments that can reduce pathogen load in commercial poultry is expected to make poultry meat safer for human consumption (Fanelli et al. 2015).

5.2.2.5 Bacteriocins

Bacteriocins constitute a variety of antimicrobial peptides produced by commensal bacteria with broad or narrow host ranges (Cotter et al. 2005, Hechard and Sahl 2002, Sahin et al. 2015). These antimicrobials usually possess a positive charge and an amphipathic structure that facilitates interaction with the negatively charged bacterial membrane or other cellular receptors on the target organism (Doyle and Erickson 2012, Sang and Blecha 2008). Further, these natural metabolites are also known to play a role in modulating the microbiome of the gastrointestinal system (Svetoch and Stern 2010). For these reasons, bacteriocins and bacteriocin-producing bacteria could serve as a viable strategy to reduce *Salmonella* and *Campylobacter* colonization in poultry (Ben Lagha et al. 2017). In this regard, Stern et al. (2005)

demonstrated the ability of the bacteriocin SRCAM 602 to reduce *C. jejuni* colonization in poultry. SRCAM 602 was purified from *Paenibacillus polymyxa* isolated from chicken intestine (Svetoch et al. 2005). The bacteriocin was microencapsulated, mixed with commercial feed, and administered to chicken at a final concentration of 250 mg/kg feed. For the study, day-old chickens were inoculated with *C. jejuni*, and SRCAM-containing feed was provided starting on day 7 for 3 consecutive days. Although control birds fed with regular feed displayed high levels of *Campylobacter* colonization (6.6–8.3 log CFU/g of feces), pathogen counts were below detection limits in all the treated birds.

Similar reductions in *Campylobacter* colonization and fecal shedding were observed with other bacteriocins including OR-7 (*L. salivarius* NRRL B-30514, Stern et al. 2006), E-760 (*Enterococcus faecium* NRRL B-30745, Line et al. 2008), and E 50–52 (*E. faecium* NRRL B-30746, Svetoch et al. 2008). Furthermore, in-feed supplementation of E-760 was found to be equally effective in eliminating detectable *C. jejuni* colonization in 10-day-old chicks and market-aged birds (42-day-old, Line et al. 2008, Svetoch et al. 2008). Wang et al. (2011) reported that administration of albusin B produced by *Ruminococcus albus* 7 reduced *Salmonella* loads and improved growth performance in broiler chickens. Although effective, one of the major drawbacks associated within the use of bacteriocins is their rapid degradation in the host gut. In order to overcome this caveat, recent studies have focused on using bacteriocin-producing bacteria to favor its colonization and antimicrobial production at the target site. Toward this, Forkus et al. (2017) engineered the probiotic *Escherichia coli* Nissile 1917 to secrete microcin J25. Administration of this strain to *Salmonella*-infected turkeys resulted in rapid clearance of the pathogen from the birds. Approximately 97% lower *Salmonella* carriage was measured in the treated group versus the control. Albeit this demonstrated efficacy, a number of issues regarding the production cost, dosage, timing, and in vivo activity of the bacteriocin remain to be addressed (Ben Lagha et al. 2017).

5.2.2.6 Bacteriophages

Bacteriophages are viruses that are predatory to bacteria and occur ubiquitously in the natural environment (Hagens and Loessner 2010). *Salmonella*- and *Campylobacter*-specific phages have been isolated from different sources including poultry manure, sewage, slaughter house effluents, and broiler chicken (Grant et al. 2016, Umaraw et al. 2017, Wernicki et al. 2017). Several of these phages have been evaluated for their ability to reduce *Salmonella* and *Campylobacter* colonization in broiler chicken (Bardina et al. 2012, Fischer et al. 2013, Loc Carrillo et al. 2005, Wong et al. 2014). Fiorentin et al. (2005) administered a single dose of phage cocktail (CNPSA1, CNPSA3, and CNPSA4 – 11 log PFU) against *S*. Enteritidis PT4 and observed a 3.5 log reduction in pathogen population in the cecum. These results are in accordance with other studies that demonstrated that use of a phage cocktail at high titer was more effective in reducing pathogen loads in contrast with the application of a single phage strain (Andreatti Filho et al. 2007, O'Flynn et al. 2004).

Similarly Bigwood et al. (2009) reported that increasing phage populations to concentrations higher than that of the target pathogen significantly improved the level of *Salmonella* reduction. More recently, Bardina et al. (2012) illustrated the significance of dosing times on phage efficacy. They observed that administration of phages a few days after *Salmonella* colonization did not result in any antimicrobial effect. This was further verified by Wong et al. (2014) who demonstrated that phage administration immediately following *Salmonella* inoculation rather than after colonization resulted in significant reduction in pathogen levels to below detection limits by 24 h. These results indicate that phages in higher titer should be administered prior to pathogen exposure in order to sustain the bacterial load reduction over time (Grant et al. 2016).

The potential use of phages to control *Campylobacte*r in poultry has been investigated in multiple studies. Loc Carrillo et al. (2005) evaluated the anti-*Campylobacter* activity of CP8 and CP34 phages when orally administered to broilers colonized by *C. jejuni* (HPC5 or GIIC8). They observed that CP8 treatment significantly reduced GIIC8 populations in the ceca and lower intestine by greater than 2 log relative to the control. However, no reduction was observed with *C. jejuni* HPC5 highlighting the high host specificity of bacteriophages. In another study, Carvalho et al. (2010) demonstrated that phage treatment was more practical when administered in feed rather than via oral gavage. Additionally, the antimicrobial effect was found to be long-lasting, and *C. jejuni* populations did not regain its original count throughout the experimental period. Nonetheless, all of these studies observed that the antimicrobial effect was not sustained, following initial reduction in population; the *Campylobacter* load stabilized itself to nearly its original counts (Hermans et al. 2011). In this case, phage treatment may be successful as a pre-slaughter application to reduce cecal loads in birds prior to processing. A study by Kittler et al. (2013) administered phage through the drinking line at a commercial poultry farm a few days before slaughter. This pre-slaughter treatment resulted in a greater than 3 log reduction in *Campylobacter* populations in the ceca of treated birds, thereby demonstrating the feasibility of a phage-based pre-harvest antimicrobial hurdle. Despite their ability to control enteropathogens, the use of phage therapy has been limited because of its high host specificity and narrow spectrum of activity. Furthermore, the need for complex identification and characterization of the bacteriophage prior to its application in food animals has restricted its application (Wernicki et al. 2017).

5.2.3 Immunization Strategies

Commercial poultry are produced from pedigree lines such as Cobb® and Ross® for broilers. These lines serve as the parent/grandparent stock and are at the apex of the production pyramid (Cox and Pavic 2010). Since *Salmonella* and *Campylobacter* can be vertically transmitted from hen to egg, control of pathogen colonization is vital in the breeder population (Cox et al. 2012, Liljebjelke et al. 2005). Additionally,

it is important to keep the breeder flocks pathogen-free since colonization in these birds will result in horizontal transmission to a large number of commercial flocks. Toward controlling *Salmonella* in breeder flocks, vaccinations are becoming part of the regular food safety regimen. Furthermore, vaccines also serve as another antimicrobial hurdle to control enteric pathogens in chicks and market-age birds on the grow-out farm. Immunization refers to the administration of antibodies (passive immunity) or antigenic proteins and attenuated pathogens (active immunity) to generate pathogen-specific immune response in chickens to neutralize and eliminate colonizing *Salmonella* and *Campylobacter* (Meunier et al. 2016). These vaccines have been developed and tested under four broad categories including passive immunization, live-attenuated, inactivated, and subunit vaccines (Alali and Hofacre 2016, Desin et al. 2013, Hermans et al. 2011, Lin 2009, Meunier et al. 2016, Vandeplas et al. 2010).

The initial finding that maternal antibodies transferred via the yolk (passive immunization) provided protection against *Salmonella* colonization in chicks highlighted the potential for using passive immunity to control enteric pathogens in poultry (Hassan and Curtiss 1996). This led to investigations into the ability of hyperimmunized hens to produce pathogen-specific antibodies in large quantities and their transfer to the yolk (Schade et al. 2005). Rahimi et al. (2007) demonstrated that chicks administered yolk immunoglobulin (IgY) through drinking water showed significantly lower fecal shedding following artificial inoculation with *S*. Enteritidis compared to the control (0% versus 14%). Similarly McGruder et al. (1995) demonstrated that in ovo administration of *S*. Enteritidis-immune lymphokines provided protection to these chicks following pathogen inoculation on hatching. Transfer of IgY to chicks has also been shown to be effective against *Campylobacter*. Hermans et al. (2014) vaccinated laying hens with *C. jejuni* whole-cell lysate. Following which, the hyperimmune egg yolk was collected, mixed with the broiler feed and administered to chicks. Following artificial inoculation with the pathogen, the birds that received the treated feed had a significantly lower cecal *C. jejuni* load when compared to the control (2.9 versus 5 log CFU/g). However, passive immunity did not provide long-term protection against the pathogen, and there is a lack of knowledge on the efficacy period of this strategy.

Whole-cell vaccines contain killed or attenuated bacteria that do not possess the virulent and colonizing abilities of the pathogen. To generate killed vaccines, different methods including heat, formalin, and acetone are employed to inactivate the bacteria (Desin et al. 2013). Clifton-Hadley et al. (2002) tested a killed *S*. Enteritidis vaccine (Salevenac®) in chickens for protection against *S*. Typhimurium oral and seeder bird challenge. Vaccinated birds consistently demonstrated a significantly ($P \leq 0.05$) lower pathogen shedding compared to unvaccinated birds. Further, this vaccine also conferred protection against *S*. Enteritidis thereby providing a valuable tool to control *Salmonella* in chicken. Similarly, the POULVAC SE® vaccine consisting of three heat-killed *S*. Enteritidis phage types was administered to broiler breeders, and the progeny was challenged with *Salmonella* on the day of hatch. It was found that vaccination significantly reduced the number of *Salmonella*-positive chicks obtained from the immunized group (28%) relative to the control

(100%, Inoue et al. 2008). In the case of *Campylobacter*, administration of formalin-inactivated *C. jejuni* F1BCB was found to significantly reduce pathogen loads in the vaccinated group. Further, these birds also had a higher IgA titer in the serum and bile when compared to the unvaccinated group (Rice et al. 1997).

The use of live-attenuated vaccines provide several advantages over killed vaccines including oral administration to birds of any age and the induction of an active immune response (antibody and cell mediated) in the host. In order to promote active and sustained immune response against *Salmonella*, several vaccine strains have been tested with varying degrees of success (Cooper et al. 1994, Desin et al. 2013, Feberwee et al. 2001, Springer et al. 2011). These vaccine strains contain mutations or deletions in genes required for metabolism, virulence, or survival. For example, immunization with an *S*. Enteritidis strain impaired in the synthesis of aromatic amines resulted in lower levels of cecal colonization by the challenge strain (Cooper et al. 1994). Similarly, oral vaccination using *S*. Typhimurium strain (*cya*/*crp* double mutant) reduced cecal *Salmonella* levels by approximately 6 log CFU/g and to undetectable levels in the spleen (Hassan and Curtiss 1994). Recently, flagellar mutants of *Salmonella* including a *phoP*/*fliC* mutant were evaluated as live-attenuated vaccine in chicken (Methner et al. 2011). Oral immunization with this vaccine strain resulted in lower *Salmonella* colonization in the liver and ceca relative to the control birds. Although these immunizations provide immune protection, concerns associated with this class of vaccines include their ability to persist in chickens as well as the environment, thereby posing threat to human health, fear of reversion to virulence, and possible interference with serological testing (Desin et al. 2013, Meunier et al. 2016).

Rather than employing the whole bacterium, subunit vaccines are made of single or multiple antigens that serve as virulence factors in pathogens (Doyle and Erickson 2012, Lin 2009). The first subunit *Campylobacter* vaccine was based on the immunodominant antigen, flagellin (Widders et al. 1998). This study administered purified native flagellin as subunit vaccine and demonstrated a systemic and mucosal humoral response in birds. However, this vaccine failed to reduce *Campylobacter* loads after the challenge. Nonetheless, when fused to the B subunit of *E. coli* labile toxin and administered orally at a higher dose of 1 mg, flagellin induced a stronger immune response and led to lower *Campylobacter* counts (Khoury and Meinersmann 1995). Recently, administration of flagellin in combination with the adjuvant Montanide demonstrated a 3 log reduction in *Campylobacter* colonization when compared to the control (Neal-McKinney et al. 2014). As with *Campylobacter*, subunit vaccines against *Salmonella* have also targeted flagellar antigens. Toyota-Hanatani et al. (2009) developed a peptide inoculation containing part of the FliC protein and used it to vaccinate chicken. This vaccine reduced *Salmonella* shedding and reduced cecal load by 2 log unit relative to the control. De Buck et al. (2005) demonstrated that Type I fimbrial antigens are another good target for vaccine development. Administration of this vaccine provided protection against *Salmonella* colonization in the reproductive tract of layers. Similarly, the use of *Salmonella* outer membrane protein extract as a vaccine helped reduce *Salmonella* colonization and fecal shedding in vaccinated birds (Meenakshi et al. 1999). Despite these

advances, several hurdles remain in the development and administration of an effective vaccine. Current research is focused on identifying immunogens that would generate a strong antibody response particularly given the short life span of broiler chickens and development of cross-acting vaccines that would provide protection against different *C. jejuni* strains and *Salmonella enterica* serovars (Hermans et al. 2011, Lin 2009, Meunier et al. 2016).

5.2.4 Litter Treatment

Poultry litter refers to the absorbent material that is used to line the floor of a poultry house. It is composed of varied material including wood shavings, pine straw, peanut hulls, rice hulls, and other dry absorbent material based on local availability (Alali and Hofacre 2016, Cox and Pavic 2010). In addition to the base material, used litter also contains large amounts of bird feces, feathers, and spilled feed. Due to the fecal contamination and high moisture content, litter has been shown to harbor many undesirable bacteria including *Salmonella*, *Campylobacter*, *E. coli*, *Clostridium perfringens*, and *Staphylococcus aureus* (Roll et al. 2011). Presence of these pathogens raises concerns about flock health and consequently consumer health (Lu et al. 2003, Roll et al. 2011). In this regard several studies have demonstrated that epidemiological link between *Salmonella* isolates detected in litter contamination to broiler carcasses (Bhatia and McNabb 1980, Bhatia et al. 1979, Corry et al. 2002). This is of particular concern since litter is generally reused for several broiler flock cycles (Alali and Hofacre 2016). Additionally, Roll et al. (2011) demonstrated that *Salmonella* was found to persist on litter used to house different broiler flocks for up to six consecutive times. Therefore, in order to control pathogens including *Salmonella* and *Campylobacter* on farms, it is critical to decontaminate poultry litter prior to its reuse.

Toward this, several researchers have investigated the potential application of litter treatments to control poultry pathogens. In a study performed by Line and Bailey (2006), two commercially available litter treatments, alum (20 lb/100 ft^2; 9.07 kg/9.3 m^2) and sodium bisulfate (8–10 lb/100 ft^2; 3.63–4.54 kg/9.3 m^2), were tested to investigate their potential to control *Campylobacter* and *Salmonella* loads in commercial broilers during the grow-out period. They observed that application of two doses of sodium bisulfate, one at the start of the trial and another on day 28, helped delay the onset of *Campylobacter* colonization in broiler chicks. However, the treatments did not result in any significant reduction in *Salmonella* populations ($P > 0.05$). Similarly, incorporation of granulated sulfuric acid (100 and 150 lb/1000 ft^2; 45.36 and 68.04 kg/92.90 m^2) and sodium bisulfate (25, 50, 75, and 100 lb/1000 ft^2; 11.34, 22.68, 34.02, and 45.36 kg/92.90 m^2) as litter treatments was tested for their effect on *Salmonella* recovery (Payne et al. 2002). Application of sulfuric acid was found to reduce litter pH to 1.53–1.93 with zero recovery of

Salmonella from the treated samples when compared to the control (4.4 $\log_{10}$/sample). Likewise treatment of litter with liquid sulfuric acid (150 lb/305 ft^2) was found to reduce *Salmonella* loads in litter to below detection limits (24 and 96 h post treatment) in comparison to the control (2.4–2.7 $\log_{10}$; Williams and Macklin 2013).

Besides chemical treatment, Stringfellow et al. (2010) investigated the application of pasteurization with steam and quicklime to reduce *S.* Typhimurium in poultry litter. Although use of steam or quicklime alone led to *Salmonella* population reduction by at least 3 orders of magnitude, combination treatment was found to be the most effective. Addition of 2.5% quicklime and steam pasteurization for minimum of 30 min completely inactivated *Salmonella* (negative by enrichment). In order to investigate the potential for litter treatments to eventually reduce cecal colonization and carcass contamination, Line (2002) inoculated broilers by raising them on naturally contaminated litter. Contaminated litter for use in this study was obtained by raising broiler chicks artificially inoculated with *Salmonella* and *Campylobacter* and allowing time to shed for 6 weeks. This litter was later treated with alum (8 or 16 lb/50 ft^2; 3.62 or 7.26 kg/4.65 m^2) or sodium bisulfite (2.5 or 4 lb/50 ft^2; 1.13 or 1.81 kg/4.65 m^2) and used to raise a fresh flock of birds. Results of the study revealed that both treatments significantly ($P \leq 0.05$) reduced *Campylobacter* colonization frequency and populations in the ceca on weeks 1, 4, and 6 of sampling. Additional no *Campylobacter* was recovered from whole carcass rinse from the treatment groups. However, these treatments were found to be ineffective in reducing *Salmonella* populations.

5.2.5 *Selective Breeding for Disease Resistance*

The genetic makeup of a bird plays a crucial role in its susceptibility to *Salmonella* and *Campylobacter* colonization (Doyle and Erickson 2012, Sahin et al. 2015, Swaggerty et al. 2014). Therefore selective breeding for improved innate immune response can be exploited as an effective pre-harvest control strategy. In this regard, studies have revealed differences in *C. jejuni* and *S.* Enteritidis colonization in different chicken lines (Kaiser and Lamont 2001, Li et al. 2008, Li et al. 2010, Swaggerty et al. 2009). This difference in susceptibility was identified to be related to the difference in host immune response to pathogen exposure in the lines tested. Chicken lines less susceptible to infection were found to be associated with an upregulation in lymphocyte and T-cell activation (Li et al. 2010), increased activation of heterophils (Chiang et al. 2008), and increased expression of cytokine genes including IL-6, IL-10, GMCSF, and TGF-β-4 (Redmond et al. 2009). These results suggest that intensive selection for increased meat production could have occurred at the expense of the immune system that influences pathogen colonization. Hence, recent research has focused on modifying parameters used for progeny selection to include improved innate immune response in addition to production traits (Swaggerty et al. 2009).

5.3 Conclusion

Extensive research in pre-harvest food safety has identified diverse management and intervention strategies that are in practice or under investigation to mitigate *Salmonella* and *Campylobacter* in live birds. Nevertheless, pre-harvest reduction of foodborne pathogens at the production environment remains challenging. For example, while many of these strategies have proven effective in limited field trials, implementation in extensive trials or true commercial operations has been problematic. Moreover, given the persistence of these zoonotic agents in the production environment and along the food continuum, effective food safety interventions are critical from the farm to the end user. However, currently there is no single effective intervention that will eliminate pathogen contamination in poultry meat from farm to fork. Therefore combination treatments may be needed starting at the farm (pre-harvest) and additional interventions at subsequent stages of production (post-harvest) including transportation, processing, packaging, retail, home, and food service establishments to provide sustained reduction in contamination and ultimately decrease incidence of foodborne illnesses. Beyond the introduction of multiple hurdles, it is equally important to educate consumers on good hygienic practices including how and why it is critical to thoroughly cook poultry meat. Regardless, research on pre-harvest inventions has enabled us to better understand the sources, transmission routes, and ecology of these pathogens. Additionally, continued research and technological advancements should reveal the complex mechanisms that influence pathogen persistence thereby identifying novel critical control points that can be targeted to reduce pathogens in animal production.

References

Alali, W. Q., & Hofacre, C. L. (2016). Preharvest food safety in broiler chicken production. *Microbiology Spectrum, 4*. https://doi.org/10.1128/microbiolspec.PFS-0002-2014

Allain, V., Chemaly, M., Laisney, M. J., Rouxel, S., Quesne, S., & Le Bouquin, S. (2014). Prevalence of and risk factors for *Campylobacter* colonisation in broiler flocks at the end of the rearing period in France. *British Poultry Science, 55*, 452–459.

Anderson, P., Stanley, V. C., Grace, B., Taylor, O., Hume, M. M., & Sefton, A. E. (2005). Use of BioMos as a pre-harvest treatment in the control of *Campylobacter* in broiler chicks. In *Nutritional biotechnology in the feed and food industries, Proceedings of the 21st Annual Symposium (Suppl 1), Lexington, Kentucky, USA* (p. 63).

Andreatti Filho, R., Higgins, J., Higgins, S., Gaona, G., Wolfenden, A., Tellez, G., et al. (2007). Ability of bacteriophages isolated from different sources to reduce *Salmonella enterica* serovar Enteritidis *in vitro* and *in vivo*. *Poultry Science, 86*, 1904–1909.

Arsi, K., Donoghue, A., Venkitanarayanan, K., Kollanoor-Johny, A., Fanatico, A., Blore, P., et al. (2014). The efficacy of the natural plant extracts, thymol and carvacrol against *Campylobacter* colonization in broiler chickens. *Journal of Food Safety, 34*, 321–325.

Arsi, K., Donoghue, A. M., Woo-Ming, A., Blore, P. J., & Donoghue, D. J. (2015). Intracloacal inoculation, an effective screening method for determining the efficacy of probiotic bacterial isolates against *Campylobacter* colonization in broiler chickens. *Journal of Food Protection, 78*, 209–213.

Audisio, M. C., Oliver, G., & Apella, M. C. (2000). Protective effect of *Enterococcus faecium* J96, a potential probiotic strain, on chicks infected with *Salmonella* Pullorum. *Journal of Food Protection, 63*, 1333–1337.

Baffoni, L., Gaggia, F., Di Gioia, D., Santini, C., Mogna, L., & Biavati, B. (2012). A *Bifidobacterium*-based synbiotic product to reduce the transmission of *C. jejuni* along the poultry food chain. *International Journal of Food Microbiology, 157*, 156–161.

Baffoni, L., Gaggia, F., Garofolo, G., Di Serafino, G., Buglione, E., Di Giannatale, E., et al. (2017). Evidence of *Campylobacter jejuni* reduction in broilers with early synbiotic administration. *International Journal of Food Microbiology, 251*, 41–47.

Bailey, J. S. (1988). Integrated colonization control of *Salmonella* in poultry. *Poultry Science, 67*, 928–932.

Bailey, J. S., Blankenship, L. C., & Cox, N. A. (1991). Effect of fructooligosaccharide on *Salmonella* colonization of the chicken intestine. *Poultry Science, 70*, 2433–2438.

Bailey, J. S., Stern, N. J., Fedorka-Cray, P., Craven, S. E., Cox, N. A., Cosby, D. E., et al. (2001). Sources and movement of *Salmonella* through integrated poultry operations: A multistate epidemiological investigation. *Journal of Food Protection, 64*, 1690–1697.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils--a review. *Food and Chemical Toxicology, 46*, 446–475.

Bardina, C., Spricigo, D. A., Cortes, P., & Llagostera, M. (2012). Significance of the bacteriophage treatment schedule in reducing *Salmonella* colonization of poultry. *Applied and Environmental Microbiology, 78*, 6600–6607.

Barnhart, E. T., Caldwell, D. J., Crouch, M. C., Byrd, J. A., Corrier, D. E., & Hargis, B. M. (1999). Effect of lactose administration in drinking water prior to and during feed withdrawal on *Salmonella* recovery from broiler crops and ceca. *Poultry Science, 78*, 211–214.

Ben Lagha, A., Haas, B., Gottschalk, M., & Grenier, D. (2017). Antimicrobial potential of bacteriocins in poultry and swine production. *Veterinary Research, 48*, 22.

Berge, A. C., & Wierup, M. (2012). Nutritional strategies to combat *Salmonella* in mono-gastric food animal production. *Animal, 6*, 557–564.

Berry, E. D., & Wells, J. E. (2016). Reducing foodborne pathogen persistence and transmission in animal production environments: Challenges and opportunities. *Microbiology Spectrum, 4*. https://doi.org/10.1128/microbiolspec.PFS-0006-2014

Bhatia, T. R., & McNabb, G. D. (1980). Dissemination of *Salmonella* in broiler-chicken operations. *Avian Diseases, 24*, 616–624.

Bhatia, T. R., McNabb, G. D., Wyman, H., & Nayar, G. P. (1979). *Salmonella* isolation from litter as an indicator of flock infection and carcass contamination. *Avian Diseases, 23*, 838–847.

Bigwood, T., Hudson, J. A., & Billington, C. (2009). Influence of host and bacteriophage concentrations on the inactivation of food-borne pathogenic bacteria by two phages. *FEMS Microbiology Letters, 291*, 59–64.

Blankenship, L., Bailey, J., Cox, N., Stern, N., Brewer, R., & Williams, O. (1993). Two-step mucosal competitive exclusion flora treatment to diminish salmonellae in commercial broiler chickens. *Poultry Science, 72*, 1667–1672.

Boxall, N. S., Perkins, N. R., Marks, D., Jones, B., Fenwick, S. G., & Davies, P. R. (2003). Free available chlorine in commercial broiler chicken drinking water in New Zealand. *Journal of Food Protection, 66*, 2164–2167.

Byrd, J. A., Corrier, D. E., Hume, M. E., Bailey, R. H., Stanker, L. H., & Hargis, B. M. (1998). Effect of feed withdrawal on *Campylobacter* in the crops of market-age broiler chickens. *Avian Diseases, 42*, 802–806.

Byrd, J. A., Hargis, B. M., Caldwell, D. J., Bailey, R. H., Herron, K. L., McReynolds, J. L., et al. (2001). Effect of lactic acid administration in the drinking water during preslaughter feed withdrawal on *Salmonella* and *Campylobacter* contamination of broilers. *Poultry Science, 80*, 278–283.

Carter, A. J., Adams, M. R., Woodward, M. J., & La Ragione, R. M. (2009). Control strategies for *Salmonella* colonization of poultry: The probiotic perspective. *Food Science and Technology, 5*, 103–115.

Carvalho, C. M., Gannon, B. W., Halfhide, D. E., Santos, S. B., Hayes, C. M., Roe, J. M., et al. (2010). The *in vivo* efficacy of two administration routes of a phage cocktail to reduce numbers of *Campylobacter coli* and *Campylobacter jejuni* in chickens. *BMC Microbiology, 10*, 232.

Cean, A., Stef, L., Simiz, E., Julean, C., Dumitrescu, G., Vasile, A., et al. (2015). Effect of human isolated probiotic bacteria on preventing *Campylobacter jejuni* colonization of poultry. *Foodborne Pathogens and Disease, 12*, 122–130.

Chaveerach, P., Keuzenkamp, D. A., Urlings, H. A., Lipman, L. J., & van Knapen, F. (2002). In vitro study on the effect of organic acids on *Campylobacter jejuni/coli* populations in mixtures of water and feed. *Poultry Science, 81*, 621–628.

Chiang, H. I., Swaggerty, C. L., Kogut, M. H., Dowd, S. E., Li, X., Pevzner, I. Y., et al. (2008). Gene expression profiling in chicken heterophils with *Salmonella* Enteritidis stimulation using a chicken 44 K Agilent microarray. *BMC Genomics, 9*, 526.

Choi, K., Namkung, H., & Paik, I. (1994). Effects of dietary fructooligosaccharides on the suppression of intestinal colonization of *Salmonella* Typhimurium in broiler chickens. *Korean Journal of Animal Science (Korea Republic), 36,* 271–284.

Clifton-Hadley, F., Breslin, M., Venables, L., Sprigings, K., Cooles, S., Houghton, S., et al. (2002). A laboratory study of an inactivated bivalent iron restricted *Salmonella enterica* serovars Enteritidis and Typhimurium dual vaccine against Typhimurium challenge in chickens. *Veterinary Microbiology, 89*, 167–179.

Collins, M. D., & Gibson, G. R. (1999). Probiotics, prebiotics, and synbiotics: Approaches for modulating the microbial ecology of the gut. *The American Journal of Clinical Nutrition, 69*, 1052S–1057S.

Cooper, G. L., Venables, L. M., Woodward, M. J., & Hormaeche, C. E. (1994). Vaccination of chickens with strain CVL30, a genetically defined *Salmonella* Enteritidis *aroA* live oral vaccine candidate. *Infection and Immunity, 62*, 4747–4754.

Corrier, D. E., Hinton Jr., A., Ziprin, R. L., & DeLoach, J. R. (1990). Effect of dietary lactose on *Salmonella* colonization of market-age broiler chickens. *Avian Diseases, 34*, 668–676.

Corry, J., Allen, V., Hudson, W., Breslin, M., & Davies, R. (2002). Sources of *Salmonella* on broiler carcasses during transportation and processing: Modes of contamination and methods of control. *Journal of Applied Microbiology, 92*, 424–432.

Cotter, P. D., Hill, C., & Ross, R. P. (2005). Bacteriocins: Developing innate immunity for food. *Nature Reviews Microbiology, 3*, 777–788.

Cox, J. M., & Pavic, A. (2010). Advances in enteropathogen control in poultry production. *Journal of Applied Microbiology, 108*, 745–755.

Cox, N. A., Berrang, M. E., & Cason, J. A. (2000). *Salmonella* penetration of egg shells and proliferation in broiler hatching eggs--a review. *Poultry Science, 79*, 1571–1574.

Cox, N. A., Richardson, L. J., Maurer, J. J., Berrang, M. E., Fedorka-Cray, P. J., Buhr, R. J., et al. (2012). Evidence for horizontal and vertical transmission in *Campylobacter* passage from hen to her progeny. *Journal of Food Protection, 75*, 1896–1902.

Darre, M., Kollanoor-Johny, A., Venkitanarayanan, K., & Upadhyaya, I. (2014). Practical implications of plant-derived antimicrobials in poultry diets for the control of *Salmonella* Enteritidis. *Journal of Applied Poultry Research, 23*, 340–344.

De Buck, J., Van Immerseel, F., Haesebrouck, F., & Ducatelle, R. (2005). Protection of laying hens against *Salmonella* Enteritidis by immunization with type 1 fimbriae. *Veterinary Microbiology, 105*, 93–101.

DeLoach, J. R., Oyofo, B. A., Corrier, D. E., Kubena, L. F., Ziprin, R. L., & Norman, J. O. (1990). Reduction of *Salmonella* Typhimurium concentration in broiler chickens by milk or whey. *Avian Diseases, 34*, 389–392.

Desin, T. S., Koster, W., & Potter, A. A. (2013). *Salmonella* vaccines in poultry: Past, present and future. *Expert Review of Vaccines, 12*, 87–96.

Diaz-Sanchez, S., D'Souza, D., Biswas, D., & Hanning, I. (2015). Botanical alternatives to antibiotics for use in organic poultry production. *Poultry Science, 94*, 1419–1430.

Doyle, M. P., & Erickson, M. C. (2012). Opportunities for mitigating pathogen contamination during on-farm food production. *International Journal of Food Microbiology, 152*, 54–74.

Durant, J. A., Corrier, D. E., & Ricke, S. C. (2000). Short-chain volatile fatty acids modulate the expression of the *hilA* and *invF* genes of *Salmonella* Typhimurium. *Journal of Food Protection, 63*, 573–578.

Epps, S. V., Petrujkic, B. T., Sedej, I., Krueger, N. A., Harvey, R. B., Beier, R. C., et al. (2015). Comparison of anti-*Campylobacter* activity of free thymol and thymol-beta-D-glucopyranoside in absence or presence of beta-glycoside-hydrolysing gut bacteria. *Food Chemistry, 173*, 92–98.

Evans, S. J., & Sayers, A. R. (2000). A longitudinal study of *Campylobacter* infection of broiler flocks in Great Britain. *Preventive Veterinary Medicine, 46*, 209–223.

Fanelli, A., Agazzi, A., Alborali, G. L., Pilotto, A., Bontempo, V., Dell'Orto, V., et al. (2015). Prevalence reduction of pathogens in poultry fed with *Saccharomyces cerevisiae*. *Biotechnologie, Agronomie, Société et Environnement, 19*, 3.

Feberwee, A., de Vries, T. S., Hartman, E. G., de Wit, J. J., Elbers, A. R., & de Jong, W. A. (2001). Vaccination against *Salmonella* Enteritidis in Dutch commercial layer flocks with a vaccine based on a live *Salmonella* Gallinarum 9R strain: Evaluation of efficacy, safety, and performance of serologic *Salmonella* tests. *Avian Diseases, 45*, 83–91.

Fernandez, F., Hinton, M., & Van Gils, B. (2000). Evaluation of the effect of mannan-oligosaccharides on the competitive exclusion of *Salmonella* Enteritidis colonization in broiler chicks. *Avian Pathology, 29*, 575–581.

Fernandez, F., Hinton, M., & Van Gils, B. (2002). Dietary mannan-oligosaccharides and their effect on chicken caecal microflora in relation to *Salmonella* Enteritidis colonization. *Avian Pathology, 31*, 49–58.

Fiorentin, L., Vieira, N. D., & Barioni Jr., W. (2005). Oral treatment with bacteriophages reduces the concentration of *Salmonella* Enteritidis PT4 in caecal contents of broilers. *Avian Pathology, 34*, 258–263.

Fischer, S., Kittler, S., Klein, G., & Glunder, G. (2013). Impact of a single phage and a phage cocktail application in broilers on reduction of *Campylobacter jejuni* and development of resistance. *PLoS One, 8*, e78543.

Forkus, B., Ritter, S., Vlysidis, M., Geldart, K., & Kaznessis, Y. N. (2017). Antimicrobial probiotics reduce *Salmonella enterica* in Turkey gastrointestinal tracts. *Scientific Reports, 7*, 40695.

Gaggia, F., Mattarelli, P., & Biavati, B. (2010). Probiotics and prebiotics in animal feeding for safe food production. *International Journal of Food Microbiology, 141*(Suppl 1), S15–S28.

Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Hautefort, I., Thompson, A., et al. (2006). Butyrate specifically down-regulates *Salmonella* pathogenicity island 1 gene expression. *Applied and Environmental Microbiology, 72*, 946–949.

Gharib Naseri, K., Rahimi, S., & Khaki, P. (2012). Comparison of the effects of probiotic, organic acid and medicinal plant on *Campylobacter jejuni* challenged broiler chickens. *Journal of Agricultural Science and Technology, 14*, 1485–1496.

Goren, E., De Jong, W., Doornenbal, P., Bolder, N., Mulder, R., & Jansen, A. (1988). Reduction of *Salmonella* infection of broilers by spray application of intestinal microflora: A longitudinal study. *The Veterinary Quarterly, 10*, 249–255.

Goren, E., De Jong, W., Doornenbal, P., Koopman, J., & Kennis, H. (1984). Protection of chicks against *Salmonella* infection induced by spray application of intestinal microflora in the hatchery. *The Veterinary Quarterly, 6*, 73–79.

Grant, A., Hashem, F., & Parveen, S. (2016). *Salmonella* and *Campylobacter*: Antimicrobial resistance and bacteriophage control in poultry. *Food Microbiology, 53*, 104–109.

Gregory, E., Barnhart, H., Dreesen, D. W., Stern, N. J., & Corn, J. L. (1997). Epidemiological study of *Campylobacter* spp. in broilers: Source, time of colonization, and prevalence. *Avian Diseases, 41*, 890–898.

Grimes, J. L., Rahimi, S., Oviedo, E., Sheldon, B. W., & Santos, F. B. (2008). Effects of a direct-fed microbial (primalac) on Turkey poult performance and susceptibility to oral *Salmonella* challenge. *Poultry Science, 87*, 1464–1470.

Grizard, D., & Barthomeuf, C. (1999). Non-digestible oligosaccharides used as prebiotic agents: Mode of production and beneficial effects on animal and human health. *Reproduction Nutrition Development, 39*, 563–588.

Gusils, C., Chaia, A. P., Gonzalez, S., & Oliver, G. (1999). Lactobacilli isolated from chicken intestines: Potential use as probiotics. *Journal of Food Protection, 62*, 252–256.

Hagens, S., & Loessner, M. J. (2010). Bacteriophage for biocontrol of foodborne pathogens: Calculations and considerations. *Current Pharmaceutical Biotechnology, 11*, 58–68.

Hassan, J. O., & Curtiss 3rd, R. (1994). Development and evaluation of an experimental vaccination program using a live avirulent *Salmonella* Typhimurium strain to protect immunized chickens against challenge with homologous and heterologous *Salmonella* serotypes. *Infection and Immunity, 62*, 5519–5527.

Hassan, J. O., & Curtiss 3rd, R. (1996). Effect of vaccination of hens with an avirulent strain of *Salmonella* Typhimurium on immunity of progeny challenged with wild-type *Salmonella* strains. *Infection and Immunity, 64*, 938–944.

Hechard, Y., & Sahl, H. G. (2002). Mode of action of modified and unmodified bacteriocins from Gram-positive bacteria. *Biochimie, 84*, 545–557.

Hermans, D., Van Deun, K., Messens, W., Martel, A., Van Immerseel, F., Haesebrouck, F., et al. (2011). *Campylobacter* control in poultry by current intervention measures ineffective: Urgent need for intensified fundamental research. *Veterinary Microbiology, 152*, 219–228.

Hermans, D., Van Steendam, K., Verbrugghe, E., Verlinden, M., Martel, A., Seliwiorstow, T., et al. (2014). Passive immunization to reduce *Campylobacter jejuni* colonization and transmission in broiler chickens. *Veterinary Research, 45*, 27.

Hinton Jr., A., Corrier, D. E., Spates, G. E., Norman, J. O., Ziprin, R. L., Beier, R. C., et al. (1990). Biological control of *Salmonella* Typhimurium in young chickens. *Avian Diseases, 34*, 626–633.

Hinton Jr., A., Corrier, D. E., Ziprin, R. L., Spates, G. E., & Deloach, J. R. (1991). Comparison of the efficacy of cultures of cecal anaerobes as inocula to reduce *Salmonella* Typhimurium colonization in chicks with or without dietary lactose. *Poultry Science, 70*, 67–73.

Hinton, M., Linton, A. H., & Perry, F. G. (1985). Control of *Salmonella* by acid disinfection of chicks' food. *The Veterinary Record, 116*, 502.

Humphrey, T. J., Henley, A., & Lanning, D. G. (1993). The colonization of broiler chickens with *Campylobacter jejuni*: Some epidemiological investigations. *Epidemiology and Infection, 110*, 601–607.

Humphrey, T. J., & Lanning, D. G. (1988). The vertical transmission of *Salmonella*s and formic acid treatment of chicken feed. A possible strategy for control. *Epidemiology and Infection, 100*, 43–49.

Iba, A. M., & Berchieri Jr., A. (1995). Studies on the use of a formic acid-propionic acid mixture (bio-add) to control experimental *Salmonella* infection in broiler chickens. *Avian Pathology, 24*, 303–311.

Inoue, A. Y., Berchieri, A. J., Bernardino, A., Paiva, J. B., & Sterzo, E. V. (2008). Passive immunity of progeny from broiler breeders vaccinated with oil-emulsion bacterin against *Salmonella* Enteritidis. *Avian Diseases, 52*, 567–571.

Jacob, J., & Pescatore A. Gut health and organic acids, antimicrobial peptides, and botanicals as natural feed additives. Organic Meat Production and Processing. Eds: Ricke SC, Van Loo EJ, Johnson MG, O'Bryan CA. 2012, Oxford, UK: Wiley-Blackwell, 351-378. 10.1002/9781118229088.ch21.

Johny, A. K., Baskaran, S. A., Charles, A. S., Amalaradjou, M. A., Darre, M. J., Khan, M. I., et al. (2009). Prophylactic supplementation of caprylic acid in feed reduces *Salmonella* enteritidis colonization in commercial broiler chicks. *Journal of Food Protection, 72*, 722–727.

Johny, A. K., Darre, M., Hoagland, T., Schreiber, D., Donoghue, A., Donoghue, D., et al. (2008). Antibacterial effect of trans-cinnamaldehyde on *Salmonella* Enteritidis and *Campylobacter jejuni* in chicken drinking water. *Journal of Applied Poultry Research, 17*, 490–497.

Jones, F. (2011). A review of practical *Salmonella* control measures in animal feed. *Journal of Applied Poultry Research, 20*, 102–113.

Kaiser, M. G., & Lamont, S. J. (2001). Genetic line differences in survival and pathogen load in young layer chicks after *Salmonella enterica* serovar Enteritidis exposure. *Poultry Science, 80*, 1105–1108.

Kerr, A. K., Farrar, A. M., Waddell, L. A., Wilkins, W., Wilhelm, B. J., Bucher, O., et al. (2013). A systematic review-meta-analysis and meta-regression on the effect of selected competitive exclusion products on *Salmonella* spp. prevalence and concentration in broiler chickens. *Preventive Veterinary Medicine, 111*, 112–125.

Khoury, C. A., & Meinersmann, R. J. (1995). A genetic hybrid of the *Campylobacter jejuni flaA* gene with LT-B of *Escherichia coli* and assessment of the efficacy of the hybrid protein as an oral chicken vaccine. *Avian Diseases, 39*, 812–820.

Kittler, S., Fischer, S., Abdulmawjood, A., Glunder, G., & Klein, G. (2013). Effect of bacteriophage application on *Campylobacter jejuni* loads in commercial broiler flocks. *Applied and Environmental Microbiology, 79*, 7525–7533.

Kollanoor Johny, A., Darre, M., Donoghue, A., Donoghue, D., & Venkitanarayanan, K. (2010). Antibacterial effect of trans-cinnamaldehyde, eugenol, carvacrol, and thymol on *Salmonella* Enteritidis and *Campylobacter jejuni* in chicken cecal contents *in vitro*. *Journal of Applied Poultry Research, 19*, 237–244.

Kollanoor-Johny, A., Mattson, T., Baskaran, S. A., Amalaradjou, M. A., Babapoor, S., March, B., et al. (2012). Reduction of *Salmonella enterica* serovar Enteritidis colonization in 20-day-old broiler chickens by the plant-derived compounds trans-cinnamaldehyde and eugenol. *Applied and Environmental Microbiology, 78*, 2981–2987.

Kurekci, C., Al Jassim, R., Hassan, E., Bishop-Hurley, S. L., Padmanabha, J., & McSweeney, C. S. (2014). Effects of feeding plant-derived agents on the colonization of *Campylobacter jejuni* in broiler chickens. *Poultry Science, 93*, 2337–2346.

Lahellec, C., & Colin, P. (1985). Relationship between serotypes of *Salmonella*e from hatcheries and rearing farms and those from processed poultry carcases. *British Poultry Science, 26*, 179–186.

Le Bouquin, S., Allain, V., Rouxel, S., Petetin, I., Picherot, M., Michel, V., et al. (2010). Prevalence and risk factors for *Salmonella* spp. contamination in French broiler-chicken flocks at the end of the rearing period. *Preventive Veterinary Medicine, 97*, 245–251.

Lee, M. D., & Newell, D. G. (2006). *Campylobacter* in poultry: Filling an ecological niche. *Avian Diseases, 50*, 1–9.

Li, X., Swaggerty, C. L., Kogut, M. H., Chiang, H., Wang, Y., Genovese, K. J., et al. (2008). The paternal effect of *Campylobacter jejuni* colonization in ceca in broilers. *Poultry Science, 87*, 1742–1747.

Li, X., Swaggerty, C. L., Kogut, M. H., Chiang, H. I., Wang, Y., Genovese, K. J., et al. (2010). Gene expression profiling of the local cecal response of genetic chicken lines that differ in their susceptibility to *Campylobacter jejuni* colonization. *PLoS One, 5*, e11827.

Liljebjelke, K. A., Hofacre, C. L., Liu, T., White, D. G., Ayers, S., Young, S., et al. (2005). Vertical and horizontal transmission of *Salmonella* within integrated broiler production system. *Foodborne Pathogens and Disease, 2*, 90–102.

Lin, J. (2009). Novel approaches for *Campylobacter* control in poultry. *Foodborne Pathogens and Disease, 6*, 755–765.

Line, J. (2002). *Campylobacter* and *Salmonella* populations associated with chickens raised on acidified litter. *Poultry Science, 81*, 1473–1477.

Line, J. E., & Bailey, J. S. (2006). Effect of on-farm litter acidification treatments on *Campylobacter* and *Salmonella* populations in commercial broiler houses in Northeast Georgia. *Poultry Science, 85*, 1529–1534.

Line, J. E., Bailey, J. S., Cox, N. A., Stern, N. J., & Tompkins, T. (1998). Effect of yeast-supplemented feed on *Salmonella* and *Campylobacter* populations in broilers. *Poultry Science, 77*, 405–410.

Line, J. E., Svetoch, E. A., Eruslanov, B. V., Perelygin, V. V., Mitsevich, E. V., Mitsevich, I. P., et al. (2008). Isolation and purification of enterocin E-760 with broad antimicrobial activity against Gram-positive and Gram-negative bacteria. *Antimicrobial Agents and Chemotherapy, 52*, 1094–1100.

Loc Carrillo, C., Atterbury, R. J., el-Shibiny, A., Connerton, P. L., Dillon, E., Scott, A., et al. (2005). Bacteriophage therapy to reduce *Campylobacter jejuni* colonization of broiler chickens. *Applied and Environmental Microbiology, 71*, 6554–6563.

Lu, J., Sanchez, S., Hofacre, C., Maurer, J. J., Harmon, B. G., & Lee, M. D. (2003). Evaluation of broiler litter with reference to the microbial composition as assessed by using 16S rRNA and functional gene markers. *Applied and Environmental Microbiology, 69*, 901–908.

Maciorowski, K. G., Jones, F. T., Pillai, S. D., & Ricke, S. C. (2004). Incidence, sources, and control of food-borne *Salmonella* spp. in poultry feeds. *World's Poultry Science Journal, 60*, 446–457.

Marin, C., Balasch, S., Vega, S., & Lainez, M. (2011). Sources of *Salmonella* contamination during broiler production in Eastern Spain. *Preventive Veterinary Medicine, 98*, 39–45.

Mathews, K. (2014). Increased dressed weights mitigate effects of reduced cattle and hog slaughter. In *Livestock and, dairy and poultry outlook* (pp. 1–23). Washington, DC: United States Department of Agriculture – Economic Research Service.

McGruder, E. D., Ramirez, G. A., Kogut, M. H., Moore, R. W., Corrier, D. E., Deloach, J. R., et al. (1995). In ovo administration of *Salmonella* Enteritidis-immune lymphokines confers protection to neonatal chicks against *Salmonella* Enteritidis organ infectivity. *Poultry Science, 74*, 18–25.

Meenakshi, M., Bakshi, C. S., Butchaiah, G., Bansal, M. P., Siddiqui, M. Z., & Singh, V. P. (1999). Adjuvanted outer membrane protein vaccine protects poultry against infection with *Salmonella* Enteritidis. *Veterinary Research Communications, 23*, 81–90.

Methner, U., Barrow, P. A., Berndt, A., & Rychlik, I. (2011). *Salmonella* Enteritidis with double deletion in phoPfliC--a potential live *Salmonella* vaccine candidate with novel characteristics for use in chickens. *Vaccine, 29*, 3248–3253.

Meunier, M., Guyard-Nicodeme, M., Dory, D., & Chemaly, M. (2016). Control strategies against *Campylobacter* at the poultry production level: Biosecurity measures, feed additives and vaccination. *Journal of Applied Microbiology, 120*, 1139–1173.

Mountzouris, K., Balaskas, C., Xanthakos, I., Tzivinikou, A., & Fegeros, K. (2009). Effects of a multi-species probiotic on biomarkers of competitive exclusion efficacy in broilers challenged with *Salmonella* enteritidis. *British Poultry Science, 50*, 467–478.

Neal-McKinney, J. M., Samuelson, D. R., Eucker, T. P., Nissen, M. S., Crespo, R., & Konkel, M. E. (2014). Reducing *Campylobacter jejuni* colonization of poultry via vaccination. *PLoS One, 9*, e114254.

Nisbet, D. (2002). Defined competitive exclusion cultures in the prevention of enteropathogen colonisation in poultry and swine. *Antonie Van Leeuwenhoek, 81*, 481–486.

Nisbet, D. J., Corrier, D. E., Scanlan, C. M., Hollister, A. G., Beier, R. C., & DeLoach, J. R. (1993). Effect of a defined continuous-flow derived bacterial culture and dietary lactose on *Salmonella* Typhimurium colonization in broiler chickens. *Avian Diseases, 37*, 1017–1025.

O'Flynn, G., Ross, R. P., Fitzgerald, G. F., & Coffey, A. (2004). Evaluation of a cocktail of three bacteriophages for biocontrol of *Escherichia coli* O157:H7. *Applied and Environmental Microbiology, 70*, 3417–3424.

Oliver, S. P., Boor, K. J., Murphy, S. C., & Murinda, S. E. (2009). Food safety hazards associated with consumption of raw milk. *Foodborne Pathogens and Disease, 6*, 793–806.

Orndorff, B. W., Novak, C. L., Pierson, F. W., Caldwell, D. J., & McElroy, A. P. (2005). Comparison of prophylactic or therapeutic dietary administration of capsaicin for reduction of *Salmonella* in broiler chickens. *Avian Diseases, 49*, 527–533.

Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V., Braden, C. R., Angulo, F. J., et al. (2013). Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerging Infectious Diseases, 19*, 407–415.

Patterson, J. A., & Burkholder, K. M. (2003). Application of prebiotics and probiotics in poultry production. *Poultry Science, 82*, 627–631.

Payne, J., Kroger, E., & Watkins, S. (2002). Evaluation of litter treatments on *Salmonella* recovery from poultry litter. *Journal of Applied Poultry Research, 11*, 239–243.

Pivnick, H., & Nurmi, E. (1982). Nurmi concept and its role in the control of *Salmonella*e in poultry. In R. Davies (Ed.), *Developments in food microbiology* (Vol. 1, pp. 41–70). London: Applied Science Publishers.

Rahimi, S., Shiraz, Z. M., Salehi, T. Z., Torshizi, M. A. K., & Grimes, J. L. (2007). Prevention of *Salmonella* infection in poultry by specific egg-derived antibody. *International Journal of Poultry Science, 6*, 230–235.

Rajan, K., Shi, Z., & Ricke, S. C. (2017). Current aspects of *Salmonella* contamination in the US poultry production chain and the potential application of risk strategies in understanding emerging hazards. *Critical Reviews in Microbiology, 43*, 370–392.

Ramirez, G. A., Sarlin, L. L., Caldwell, D. J., Yezak Jr., C. R., Hume, M. E., Corrier, D. E., et al. (1997). Effect of feed withdrawal on the incidence of *Salmonella* in the crops and ceca of market age broiler chickens. *Poultry Science, 76*, 654–656.

Rantala, M., & Nurmi, E. (1973). Prevention of the growth of *Salmonella* Infantis in chicks by the flora of the alimentary tract of chickens. *British Poultry Science, 14*, 627–630.

Redmond, S. B., Chuammitri, P., Andreasen, C. B., Palic, D., & Lamont, S. J. (2009). Chicken heterophils from commercially selected and non-selected genetic lines express cytokines differently after *in vitro* exposure to *Salmonella* Enteritidis. *Veterinary Immunology and Immunopathology, 132*, 129–134.

Rehman, H., Vahjen, W., Kohl-Parisini, A., Ijaz, A., & Zentek, J. (2009). Influence of fermentable carbohydrates on the intestinal bacteria and enteropathogens in broilers. *World's Poultry Science Journal, 65*, 75–90.

Reichling, J., Schnitzler, P., Suschke, U., & Saller, R. (2009). Essential oils of aromatic plants with antibacterial, antifungal, antiviral, and cytotoxic properties--an overview. *Forschende Komplementärmedizin, 16*, 79–90.

Rice, B. E., Rollins, D. M., Mallinson, E. T., Carr, L., & Joseph, S. W. (1997). Campylobacter jejuni in broiler chickens: Colonization and humoral immunity following oral vaccination and experimental infection. *Vaccine, 15*, 1922–1932.

Ricke, S. C. (2003). Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry Science, 82*, 632–639.

Ricke, S. C. (2015). Potential of fructooligosaccharide prebiotics in alternative and nonconventional poultry production systems. *Poultry Science, 94*, 1411–1418.

Roche, A. J., Cox, N. A., Richardson, L. J., Buhr, R. J., Cason, J. A., Fairchild, B. D., et al. (2009). Transmission of *Salmonella* to broilers by contaminated larval and adult lesser mealworms, *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). *Poultry Science, 88*, 44–48.

Roll, V. F., Dai Pra, M. A., & Roll, A. P. (2011). Research on *Salmonella* in broiler litter reused for up to 14 consecutive flocks. *Poultry Science, 90*, 2257–2262.

Sahin, O., Kassem, I. I., Shen, Z., Lin, J., Rajashekara, G., & Zhang, Q. (2015). *Campylobacter* in poultry: Ecology and potential interventions. *Avian Diseases, 59*, 185–200.

Saint-Cyr, M. J., Guyard-Nicodeme, M., Messaoudi, S., Chemaly, M., Cappelier, J. M., Dousset, X., et al. (2016). Recent advances in screening of anti-*Campylobacter* activity in probiotics for use in poultry. *Frontiers in Microbiology, 7*, 553.

Sang, Y., & Blecha, F. (2008). Antimicrobial peptides and bacteriocins: Alternatives to traditional antibiotics. *Animal Health Research Reviews, 9*, 227–235.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., et al. (2011). Foodborne illness acquired in the United States--major pathogens. *Emerging Infectious Diseases, 17*, 7–15.

Schade, R., Calzado, E. G., Sarmiento, R., Chacana, P. A., Porankiewicz-Asplund, J., & Terzolo, H. R. (2005). Chicken egg yolk antibodies (IgY-technology): A review of progress in production and use in research and human and veterinary medicine. *Alternatives to Laboratory Animals, 33*, 129–154.

Schrezenmeir, J., & de Vrese, M. (2001). Probiotics, prebiotics, and synbiotics--approaching a definition. *The American Journal of Clinical Nutrition, 73*, 361S–364S.

Skanseng, B., Kaldhusdal, M., Moen, B., Gjevre, A. G., Johannessen, G. S., Sekelja, M., et al. (2010). Prevention of intestinal *Campylobacter jejuni* colonization in broilers by combinations of in-feed organic acids. *Journal of Applied Microbiology, 109*, 1265–1273.

Skov, M. N., Madsen, J. J., Rahbek, C., Lodal, J., Jespersen, J. B., Jorgensen, J. C., et al. (2008). Transmission of *Salmonella* between wildlife and meat-production animals in Denmark. *Journal of Applied Microbiology, 105*, 1558–1568.

Solis de Los Santos, F., Donoghue, A. M., Venkitanarayanan, K., Dirain, M. L., Reyes-Herrera, I., Blore, P. J., et al. (2008a). Caprylic acid supplemented in feed reduces enteric *Campylobacter jejuni* colonization in ten-day-old broiler chickens. *Poultry Science, 87*, 800–804.

Solis de Los Santos, F., Donoghue, A. M., Venkitanarayanan, K., Reyes-Herrera, I., Metcalf, J. H., Dirain, M. L., et al. (2008b). Therapeutic supplementation of caprylic acid in feed reduces *Campylobacter jejuni* colonization in broiler chicks. *Applied and Environmental Microbiology, 74*, 4564–4566.

Spring, P., Wenk, C., Dawson, K. A., & Newman, K. E. (2000). The effects of dietary mannaoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of *Salmonella*-challenged broiler chicks. *Poultry Science, 79*, 205–211.

Springer, S., Lindner, T., Ahrens, M., Woitow, G., Prandini, F., & Selbitz, H. J. (2011). Duration of immunity induced in chickens by an attenuated live *Salmonella* Enteritidis vaccine and an inactivated *Salmonella* Enteritidis/Typhimurium vaccine. *Berliner und Münchener Tierärztliche Wochenschrift, 124*, 89–93.

Stern, N. J., Fedorka-Cray, P., Bailey, J. S., Cox, N. A., Craven, S. E., Hiett, K. L., et al. (2001). Distribution of *Campylobacter* spp. in selected U.S. poultry production and processing operations. *Journal of Food Protection, 64*, 1705–1710.

Stern, N. J., Svetoch, E. A., Eruslanov, B. V., Kovalev, Y. N., Volodina, L. I., Perelygin, V. V., et al. (2005). *Paenibacillus polymyxa* purified bacteriocin to control *Campylobacter jejuni* in chickens. *Journal of Food Protection, 68*, 1450–1453.

Stern, N. J., Svetoch, E. A., Eruslanov, B. V., Perelygin, V. V., Mitsevich, E. V., Mitsevich, I. P., et al. (2006). Isolation of a *Lactobacillus salivarius* strain and purification of its bacteriocin, which is inhibitory to *Campylobacter jejuni* in the chicken gastrointestinal system. *Antimicrobial Agents and Chemotherapy, 50*, 3111–3116.

Stringfellow, K., Caldwell, D., Lee, J., Byrd, A., Carey, J., Kessler, K., et al. (2010). Pasteurization of chicken litter with steam and quicklime to reduce *Salmonella* Typhimurium. *Journal of Applied Poultry Research, 19*, 380–386.

Šušković, J., Kos, B., Goreta, J., & Matošić, S. (2001). Role of lactic acid bacteria and bifidobacteria in synbiotic effect. *Food Technology and Biotechnology, 39*, 227–235.

Svetoch, E. A., Eruslanov, B. V., Perelygin, V. V., Mitsevich, E. V., Mitsevich, I. P., Borzenkov, V. N., et al. (2008). Diverse antimicrobial killing by *Enterococcus faecium* E 50-52 bacteriocin. *Journal of Agricultural and Food Chemistry, 56*, 1942–1948.

Svetoch, E. A., & Stern, N. J. (2010). Bacteriocins to control *Campylobacter* spp. in poultry--A review. *Poultry Science, 89*, 1763–1768.

Svetoch, E. A., Stern, N. J., Eruslanov, B. V., Kovalev, Y. N., Volodina, L. I., Perelygin, V. V., et al. (2005). Isolation of *Bacillus circulans* and *Paenibacillus polymyxa* strains inhibitory to *Campylobacter jejuni* and characterization of associated bacteriocins. *Journal of Food Protection, 68*, 11–17.

Swaggerty, C. L., Pevzner, I. Y., He, H., Genovese, K. J., Nisbet, D. J., Kaiser, P., et al. (2009). Selection of broilers with improved innate immune responsiveness to reduce on-farm infection by foodborne pathogens. *Foodborne Pathogens and Disease, 6*, 777–783.

Swaggerty, C. L., Pevzner, I. Y., & Kogut, M. H. (2014). Selection for pro-inflammatory mediators yields chickens with increased resistance against *Salmonella enterica* serovar Enteritidis. *Poultry Science, 93*, 535–544.

Tellez, G. I., Jaeger, L., Dean, C. E., Corrier, D. E., DeLoach, J. R., Williams, J. D., et al. (1993). Effect of prolonged administration of dietary capsaicin on *Salmonella* Enteritidis infection in leghorn chicks. *Avian Diseases, 37*, 143–148.

Thitaram, S. N., Chung, C. H., Day, D. F., Hinton Jr., A., Bailey, J. S., & Siragusa, G. R. (2005). Isomaltooligosaccharide increases cecal *Bifidobacterium* population in young broiler chickens. *Poultry Science, 84*, 998–1003.

Thompson, J. L., & Hinton, M. (1997). Antibacterial activity of formic and propionic acids in the diet of hens on *Salmonella*s in the crop. *British Poultry Science, 38*, 59–65.

Torrence, M. E. (2016). Introduction to preharvest food safety. *Microbiology Spectrum, 4*. https://doi.org/10.1128/microbiolspec.PFS-0009-2015

Totton, S. C., Farrar, A. M., Wilkins, W., Bucher, O., Waddell, L. A., Wilhelm, B. J., et al. (2012). The effectiveness of selected feed and water additives for reducing *Salmonella* spp. of public health importance in broiler chickens: A systematic review, meta-analysis, and meta-regression approach. *Preventive Veterinary Medicine, 106*, 197–213.

Toyota-Hanatani, Y., Kyoumoto, Y., Baba, E., Ekawa, T., Ohta, H., Tani, H., et al. (2009). Importance of subunit vaccine antigen of major Fli C antigenic site of *Salmonella* Enteritidis II: A challenge trial. *Vaccine, 27*, 1680–1684.

Umaraw, P., Prajapati, A., Verma, A. K., Pathak, V., & Singh, V. P. (2017). Control of *Campylobacter* in poultry industry from farm to poultry processing unit: A review. *Critical Reviews in Food Science and Nutrition, 57*, 659–665.

Upadhyay, A., Upadhyaya, I., Kollanoor-Johny, A., & Venkitanarayanan, K. (2014). Combating pathogenic microorganisms using plant-derived antimicrobials: A minireview of the mechanistic basis. *BioMed Research International, 2014*, 761741.

Upadhyaya, I., Upadhyay, A., Kollanoor-Johny, A., Mooyottu, S., Baskaran, S. A., Yin, H. B., et al. (2015). In-feed supplementation of trans-cinnamaldehyde reduces layer-chicken egg-borne transmission of *Salmonella enterica* serovar Enteritidis. *Applied and Environmental Microbiology, 81*, 2985–2994.

Van Coillie, E., Goris, J., Cleenwerck, I., Grijspeerdt, K., Botteldoorn, N., Van Immerseel, F., et al. (2007). Identification of lactobacilli isolated from the cloaca and vagina of laying hens and characterization for potential use as probiotics to control *Salmonella* Enteritidis. *Journal of Applied Microbiology, 102*, 1095–1106.

Van Immerseel, F., De Buck, J., Boyen, F., Bohez, L., Pasmans, F., Volf, J., et al. (2004). Medium-chain fatty acids decrease colonization and invasion through hilA suppression shortly after infection of chickens with *Salmonella enterica* serovar Enteritidis. *Applied and Environmental Microbiology, 70*, 3582–3587.

Van Immerseel, F., Russell, J., Flythe, M., Gantois, I., Timbermont, L., Pasmans, F., et al. (2006). The use of organic acids to combat *Salmonella* in poultry: A mechanistic explanation of the efficacy. *Avian Pathology, 35*, 182–188.

Vandeplas, S., Dubois Dauphin, R., Beckers, Y., Thonart, P., & Thewis, A. (2010). *Salmonella* in chicken: Current and developing strategies to reduce contamination at farm level. *Journal of Food Protection, 73*, 774–785.

Vicente, J., Lopez, C., Avila, E., Morales, E., Hargis, B., & Tellez, G. (2007). Effect of dietary natural capsaicin on experimental *Salmonella* Enteritidis infection and yolk pigmentation in laying hens. *International Journal of Poultry Science, 6*, 393–396.

Vidanarachchi, J., Mikkelsen, L., Sims, I., Iji, P., & Choct, M. (2005). Phytobiotics: Alternatives to antibiotic growth promoters in monogastric animal feeds. *Recent Advances in Animal Nutrition in Australia, 15*, 131–144.

Wales, A., McLaren, I., Rabie, A., Gosling, R. J., Martelli, F., Sayers, R., et al. (2013). Assessment of the anti-*Salmonella* activity of commercial formulations of organic acid products. *Avian Pathology, 42*, 268–275.

Wang, H. T., Yu, C., Hsieh, Y. H., Chen, S. W., Chen, B. J., & Chen, C. Y. (2011). Effects of albusin B (a bacteriocin) of *Ruminococcus albus* 7 expressed by yeast on growth performance and intestinal absorption of broiler chickens--its potential role as an alternative to feed antibiotics. *Journal of the Science of Food and Agriculture, 91*, 2338–2343.

Wang, R. J., Li, D. F., & Bourne, S. (1998). Can 2000 years of herbal medicine history help us solve problems in the year 2000. In *Proc Alltech's Ann Symp Nottingham. Biotechnology in the Feed Industry* (pp. 273–291). Nicholasville, KY: Alltech Technical Publications.

Wedderkopp, A., Gradel, K. O., Jorgensen, J. C., & Madsen, M. (2001). Pre-harvest surveillance of *Campylobacter* and *Salmonella* in Danish broiler flocks: A 2-year study. *International Journal of Food Microbiology, 68*, 53–59.

Wenk, C. (2006). Are herbs, botanicals and other related substances adequate replacements for antimicrobial growth promoters? In D. Barug, J. de Jong, J. Kies, & V. MWA (Eds.), *Antimicrobial growth promoters* (pp. 329–340). Wageningen, The Netherlands: Wageningen Academic Publishers.

Wernicki, A., Nowaczek, A., & Urban-Chmiel, R. (2017). Bacteriophage therapy to combat bacterial infections in poultry. *Virology Journal, 14*, 179.

Widders, P. R., Thomas, L. M., Long, K. A., Tokhi, M. A., Panaccio, M., & Apos, E. (1998). The specificity of antibody in chickens immunised to reduce intestinal colonisation with *Campylobacter jejuni*. *Veterinary Microbiology, 64*, 39–50.

Williams, Z., & Macklin, K. (2013). Reduction of *Salmonella* and ammonia emissions in broiler litter using sulfuric acid and aluminum sulfate. *International Journal of Poultry Science, 12*, 328–334.

Windisch, W., Schedle, K., Plitzner, C., & Kroismayr, A. (2008). Use of phytogenic products as feed additives for swine and poultry. *Journal of Animal Science, 86*, E140–E148.

Wong, C. L., Sieo, C. C., Tan, W. S., Abdullah, N., Hair-Bejo, M., Abu, J., et al. (2014). Evaluation of a lytic bacteriophage, Phi st1, for biocontrol of *Salmonella enterica* serovar Typhimurium in chickens. *International Journal of Food Microbiology, 172*, 92–101.

Yamazaki, M., Ohtsu, H., Yakabe, Y., Kishima, M., & Abe, H. (2012). In vitro screening of lactobacilli isolated from chicken excreta to control *Salmonella* Enteritidis and Typhimurium. *British Poultry Science, 53*, 183–189.

Yang, Y., Iji, P., Kocher, A., Mikkelsen, L., & Choct, M. (2007). Effects of mannanoligosaccharide on growth performance, the development of gut microflora, and gut function of broiler chickens raised on new litter. *Journal of Applied Poultry Research, 16*, 280–288.

Yusrizal, Y., & Chen, T. C. (2003). Effect of adding chicory fructans in feed on fecal and intestinal flora. *International Journal of Poultry Science, 2*, 188–194.

Zhang, G., Ma, L., & Doyle, M. P. (2007a). Salmonellae reduction in poultry by competitive exclusion bacteria *Lactobacillus salivarius* and *Streptococcus cristatus*. *Journal of Food Protection, 70*, 874–878.

Zhang, G., Ma, L., & Doyle, M. P. (2007b). Potential competitive exclusion bacteria from poultry inhibitory to *Campylobacter jejuni* and *Salmonella*. *Journal of Food Protection, 70*, 867–873.

Chapter 6
Post-harvest Approaches to Improve Poultry Meat Safety

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6.1 Introduction

According to current health food choice, most of the people in developed countries including the USA, Canada, and many European countries prefer to consume white meat, particularly chicken meat for their protein source (Marangoni et al. 2015). Growth of yearly poultry production is now more than 3% globally, and due to the modern giant corporate animal framing and practices, the products from the same farm are now available in different parts of the same continent and multiple continents (USDA-ERS 2015). At the same time, poultry-borne zoonotic infections are rising (CDC 2013), and very often the poultry meat and poultry products are required to recall (CDC 2013). Thus, safety and quality of poultry and poultry products are of utmost importance to reduce the foodborne enteric infections. Though the safety and microbiological contamination level of poultry meat is determined by the health status of the live birds and degree of colonization with poultry-borne

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zoonotic pathogens at the pre-harvest level, a great deal of attention in post-harvest processing must be given to limiting cross-contamination at the processing plants. In general, birds arriving for slaughter are heavily contaminated with microorganisms which are carried in the intestines and on the skin and follicle of the feathers.

Further, it is almost impossible to process individual carcasses separately in a modern fully automated poultry processing plant where machines run according to preprogrammed computerized systems. Ultimately, cross-contamination occurs at any of the stages of the process if fecal samples or any other contaminant remain on a single carcass. Therefore, several precautions and modifications are essential to reduce the contamination and have been introduced while some are still in development (Barbut 2016). To reach that goal, proper poultry farming and post-harvest processing is critical, which includes slaughtering, processing and packaging, transportation and distribution, retailing, foodservice, and food preparation at home, all of which are extremely critical controls to keep foodborne infection in check.

Though the impact of modern food processing and manufacturing methods is evident in today's excellent food supply system, the safety and quality of poultry products can still be improved and/or enhanced if the post-harvest processing, packaging, and transportation can be performed properly following the integration of scientific information. Further, the dissemination of the research outcome through outreach activities can help consumers to handle the poultry, meat, and eggs wisely in the kitchen. In this chapter, we aimed to recommend the possible precautions and safety measures for better post-harvest processing of poultry products by reviewing the concurrent research articles and findings. We also focus on the possible mistakes that happen in the kitchen and recommendations for better practices.

6.2 Major Post-harvest Processing Steps

Prior to processing of poultry in the processing plant, several steps are required to prepare the live birds, including feed removal and visual screening for diseases before slaughter (Grandison 2012). Feed withdrawal is important to reduce the gastrointestinal contents within the bird, which decreases the chance of fecal contamination or cross-contamination during processing (Grandison 2012). It also increases pH of the carcasses. In an ideal processing plant, processors would like a continuous supply of raw poultry meat, whose composition and quality are consistent. Although it is almost impossible to achieve, by following proper guidelines and best hygienic practices, growers can synchronize the environment to produce the best raw poultry.

After unloading birds from delivery truck, birds are transferred into the plant to the live hang area. The whole processing and subsequent operations are shown in the Fig. 6.1. During the processing of poultry in the processing plant, water is used in several steps, and these steps possess a high risk of cross-contamination of poultry meat and poultry products with microbial pathogens. This occurs either from the fecal materials or colonized microbes on the skin and feather follicles. In addition to wet steps of processing, evisceration is a critical step in microbial contamination

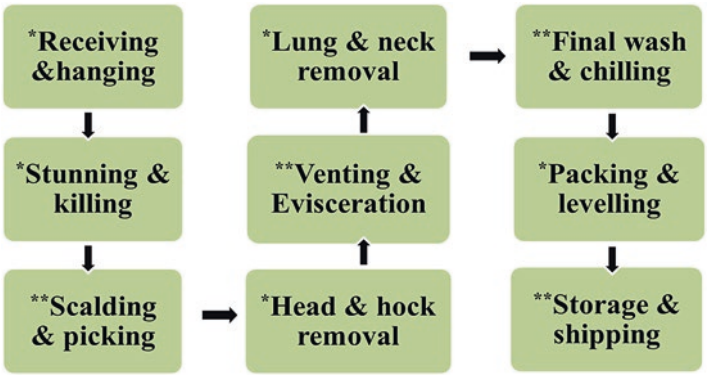

Fig. 6.1 Flowchart of the major poultry processing steps with the degree of possible risk of pathogenic microbial contamination

or cross-contamination of the carcasses. The monitoring of carcasses after evisceration is mandatory by the US Department of Agriculture (USDA) Food Safety Inspection Service (FSIS), and processing plants are required to add a physical separation or partition between slaughter and evisceration (FSIS 2014).

Employees manually pick up each bird and hang it upside down by the feet on a stainless shackle. Shackles are spaced approximately 6 in. apart and are attached to rollers on a continuous track. Birds remain on these shackles for several minutes and pass through an electrical stunning device to keep them calm during the automated killing machine, which cuts the throat of the bird. After the neck is cut, exsanguination takes a few minutes, and the carcasses are scalded in hot water to reduce the external microbial load. After scalding, the carcasses pass into picking machines to remove the feathers. The picking machines contain rows of picker fingers, which are blunt, ribbed, made of hard rubber, and approximately 4 in. long and a half inch wide. In order to remove the feathers, the picker fingers are mounted on metal plates that spin at a high speed and are horizontal to both sides of the carcass.

After lung and neck removal and visual inspection, the viscera of the carcass are separated into two portions: edible and inedible. The edible portion includes the giblets, or heart, liver, and gizzard, and the inedible portion includes the intestines, spleen, gallbladder, and others. For giblet processing, the gizzards are split open and the lining peeled away. Hearts are trimmed of aorta, and livers are put together with the gallbladder cut or peeled. These are processed separately in a smaller chilling tank or sent to the packing section for raw or frozen shipment. Then, all carcasses will be washed thoroughly, known as final wash. After the final wash, the whole carcasses are transferred to big tanks commonly known as chilling tank, where carcasses are immersed under ice-cold water containing antimicrobial components such as chlorine and organic acids. According to the USDA-FSIS, smaller broilers must be chilled to 40 °F or less within 4 h of slaughter, and larger birds have to undergo 8 h for chilling. In addition to immersion chilling, an air-chilling method is also performed at limited numbers of poultry processing plants in the USA where carcasses were generally found to be less contaminated (Table 6.1).

Table 6.1 Guidelines for cooking chicken

Cut of meat	Recommended weight	Cooking style		
		Roasted (min)	Grilled (min)	Simmered (min)
Wings	2–3 oz	30–40	8–12 (each side)	30–45
Legs or thighs	4–8 oz	40–50	10–15 (each side)	40–50
Boneless breast	4 oz	20–30	6–8 (each side)	25–30
Bone-in breast	6–8 oz	30–40	10–15 (each side)	35–45
Whole	48–64 oz	85–110	60–75	60–75

6.3 Highly Sensitive Phases/Points of Contamination

The most critical points for cross-contamination during processing of poultry carcasses are scalding and picking, venting and evisceration, and final wash and chilling. At the scalding and picking stages, many bacteria are washed from the carcasses to the water, which results in the contamination of other carcasses, if there is no proper treatment of water. Stahl (1996) pointed out that frequently spraying e carcasses between units, where the water drains back into the tank that the birds have just left, further improves the water quality. Ionization and homogenization (cell disrupters) are further applications that could be used to reduce the number of microorganisms in water. Treatment of the scalding water with organic acids (acetic or lactic) can be helpful. Additionally, removal of feathers opens the follicles, where f microbes colonize. The use of high-pressure (800 bars) water wash was shown to yield a 100-fold reduction in the number of *Enterobacteriaceae* (van der Wal and Muller 1996) on the skin. Plucking (defeathering) with automatic machinery causes considerable scattering of microorganisms, potentially exacerbating cross-contamination of carcasses. Hinton et al. (1996) and Tinker et al. (1996) reported that using separate compartment defeathering on a carousel can result in a 100-fold reduction in cross-contamination of carcasses. The use of a tail feather puller and cleansing of carcass before it passes on to be eviscerated can also be of t benefit (Stahl 1996). Spraying of warm chlorinated water on the carcasses (52 °C/45 mg/l chlorine) during and after plucking was also found to be effective in reducing cross-contamination with poultry-borne zoonotic pathogens (Stahl 1996).

Evisceration of carcasses is the most critical point in cross-contamination of poultry carcasses with fecal material and fecal microbes that result in problems to the rest of the processing steps, particularly chilling and final wash. Due to the damage of the intestines or the contact between intestines and carcasses, cross-contamination can occur. The problem of intestinal rupture is made worse when the evisceration equipment is not properly adjusted and monitored. Therefore, more adaptable machines (which adjust to variations in carcass size) are being developed. The introduction of a new and upgraded evisceration system can be effective in improving the process by opening of the carcass automatically for evisceration and transferring the intestines to a synchronously running organ line. Stahl (1996) also reported that the introductions of automatic systems for giblet harvesting and cleaning are very effective in further improving the microbial quality of carcass. In

addition, it is beneficial to wash the carcasses at different stages of processing after defeathering and before chilling. The intensive and careful use of inside-outside wash also removes visible fecal materials as well as portion of invisible microbial cells from the carcasses, but does not eliminate invisible microbes completely.

After evisceration, chicken carcasses are cooled by either cold-water immersion or cold air-blast (Brant 1974; Thomson et al. 1974; Veerkamp 1999), commonly known as chilling. Immersion chilling has traditionally been the most popular method for cooling poultry in the USA, and more than 90% of poultry processing plants practice this method. Based on research findings, immersion chilling is most effective because mechanical agitation along with air injection in chiller water produces economical and efficient carcass heat transfer and killing of microbes on the carcass. In spite of its increased efficiency, immersion chilling has been criticized as it requires large volumes of water, and in the chiller tank, cross-contamination occurs as microbes are transferred from one carcass to another in the common bath (Mead et al. 2000; James et al. 2006). Approximately 10% of poultry processing plants in the USA are practicing air chilling as an alternative to immersion chilling to eliminate carcass cross-contamination; however, research has shown that cross-contamination can still occur during both dry and evaporative air chilling (Mead et al. 2000).

6.4 Recommendation for Preventing Microbial Contamination During Post-harvest Processing

To limit or control the cross-contamination of chicken carcasses with poultry-borne pathogenic microorganisms, specifically zoonotic pathogens, and to improve the safety of finished products, the basic principles followed for Sanitation Standard Operating Procedures and Good Manufacturing Practices in Hazard analysis and critical control points and its implications through educating and training of workers are very crucial. The major microbiological safety concerns in poultry farming (pre-harvest level), poultry processing plant, and its environment (post-harvest level) are briefly discussed in the following section.

External Infection with Zoonotic/Animal Pathogens and Pre-slaughter Check The hatching and farming practices influence the quality and safety of the poultry products. Safe poultry production needs to be initiated at broiler hatcheries. According to the existing evidence, both major poultry-borne zoonotic pathogens, *Campylobacter* and *Salmonella* infections, can be linked to horizontal contamination as well as vertical infection from hens to chicks (Hafez 1999; Pearson et al. 1996). Pre-slaughter mortality or loss of chicks at the farm level has been found in Con farms where farmers did not check the quality of arriving chicks (Jacobs et al. 2017). To avoid the horizontal contamination at the hatching level, equipment and the room that are in direct contact with eggs, as well as the eggs themselves, should be thoroughly sanitized and confirmed as pathogen-free. Chicks arriving from hatcheries should also be inspected before a new flock is received by growers using newly developed quick microbiological detection methodology.

Following the delivery of a new flock, proper hygienic condition of housing is required to maintain the health of the chicks as well as safety of workers. Quality and design of housing and maintaining practices are essential to reduce the spread of infection within commercial poultry population. Rodents, wild birds, pests, and insects have been implicated in poultry infection transmission (Hafez 1999). Often, the pests and insects can be transported to the poultry through feed. Therefore, it is important to inspect the feed quality and cleanliness of the feed container and workers as well as implement hygienic storage of poultry foodstuffs. During summer months, verities of ectoparasites, especially flies and mosquitoes, play a critical role in spreading pathogens particularly *Campylobacter*, *Salmonella*, and other pathogens on broiler farms. The use of fly screens or trap has been shown to reduce the amount of flocks testing positive for poultry-borne bacterial pathogens (Food Safety Authority of Ireland 2011).

The Food Safety Authority of Ireland recommended that random screening of zoonotic pathogen load, such as *Campylobacter* and *Salmonella*, in fecal samples of chicks should be implemented at the pre-harvest level to reduce colonization. The concentration of *Campylobacter* spp. in fecal sample should be less than 10^7 CFU/g (FSIS 2014). Repeated over-limit of pre- or post-harvest concentrations of pathogens specifically *Campylobacter* and *Salmonella* requires review of sanitation practices within a facility (FSIS 2014).

Effective Antimicrobial Containing Culling Water, Duration of Treatment, and Limit of Recycling of Water Post-slaughter immersion or sub-immersion in antimicrobial containing hot water is critical to remove external poultry contamination present on the skin and feathers and loosen the skin to facilitate quick and easy feather removal. Counterflow scalding and antimicrobial water containing tanks with multiple stages can reduce pathogenic bacterial load remaining on poultry following scalding (Food Safety Authority of Ireland 2011; Sutmoller 1997; Yang et al. 2001). Yang et al. (2001) reported that counterflow scalding prevents microorganisms that were initially present from remaining and contaminating poultry at the end of the cycle. Sampling for determining pathogenic microbial load in recycling water should be done to ensure that water near the end of the cycle is under the hazardous limit than the water at the beginning of the cycle. Researchers also suggested that adding fresh water to the end of the cycle might help meet the requirements for food safety (Scott 2013). Slavik et al. (1995) reported that increasing the temperature of culling water from 56 °C to 60 °C reduced the population of both *Salmonella* and *Campylobacter*. In water tanks with scalding temperatures below 57 °C, carcasses should be treated at least 3 min or more and few minutes in scalding water above this temperature (Nunes 2011). Water flow rates should be increased as much as possible, and acidic disinfectant or appropriate antimicrobial should be added to the water. Mead et al. (2010) found that increasing the temperature of scalding water up to 75 °C during break can reduce contamination between new batches of carcasses.

Impact of Speed of Water and Intensity of Washing After Defeathering in Bacterial Load Defeathering practices have been recognized as one of the

important steps that increase cross-contamination of carcasses during poultry processing. After defeathering, there are increased hiding holes, such as bigger size of follicles, which can harbor poultry-borne pathogens such as *Campylobacter*, *Staphylococcus aureus*, *Salmonella*, and viruses (Singh et al. 2015). Nayak et al. (2001) observed that scanning electron microscopy revealed bacteria colonized deeper in broiler skin or follicles that could not be recovered by rinsing or stomaching but were recoverable by shredding. In addition, the fingers of the picker have also been identified as ideal routes for contamination since they are always in warm temperature and humid environments, and cleaning and disinfecting of fingers of the pickers are very difficult. The type and amount of organic matter present on the skin of birds that ultimately end up in the defeathering environment can determine the possible species of bacterial pathogen present and what type and/or how readily cleaning and sanitizing agents can inactivate them. Thus, evaluation of the type and amount of organic matter and the type of contaminates should be considered for setting the water speed and intensity, with or without antimicrobial supplementation.

Preventive Measure and Visual Check of Evisceration Before Chilling Taking preventive measures to control transmission of bacterial pathogens prior to evisceration of poultry carcasses can be an extremely effective step. *Salmonella* and *Campylobacter* cause the largest number of foodborne illnesses associated with poultry and poultry products (Sutmoller 1997). Evisceration is one of the most critical points of poultry processing where cross-contamination of carcasses with these bacterial pathogens occurs (Hafez 1999). Implementation of simple preventative steps can reduce the chances of contamination by these bacteria pathogens during evisceration. The maintenances of slaughtering equipment particularly alignment and calibration are imperative for good sanitation. Improper alignment of the slaughtering equipment often causes evisceration failures, which can lead to contamination and/or cross-contamination of carcasses (Food Safety Authority of Ireland 2011). Prior to evisceration, appropriate adjustment of equipment to the size of birds is essential. When evisceration failures are monitored repeatedly, that indicates improper sizing or misadjustment or dysfunctional equipment, which may need to be replaced (Food Safety Authority of Ireland 2011).

The risk of cross-contamination can be reduced by the use of antimicrobial components such as chlorinated water and trisodium phosphate dip immediately after water chilling or before air chilling (Hinton and Corry 1998). The use of a low-voltage electrical current with a low concentration of salt in the chill water has also been shown to reduce the poultry-borne zoonotic bacterial pathogens in chiller water and to reduce the contamination on chicken skin with pathogenic microbes (Li and Slavik 1996). The type of antimicrobial component and its concentration in addition to the duration of chilling and temperature of the chilling tank also reduces cross-contamination of the carcass with pathogens.

Certified inspectors, who are qualified to identify any abnormal sign of the carcasses specifically contamination with fecal material or rupture of muscle, must perform a visual check of each individual carcass on site pre- and post-chilling (Barbut 2016). The personnel inspection should also include the inside and outside

of each carcass looking for feathers/hairs, damaged internal organs, skin discolorations, exposed flesh, conformation, disjointed/broken bones, missing parts, and pathological lesions/tumors/blood clots in accordance with the USDA quality criterion (Barbut 2016). It was found that damaged internal organs could increase possibility of fecal matter contaminated carcass number and render it condemnable. Once certified inspectors examine each carcass and then they declare carefully as "passed", "trimmed/salvaged/washed passed", "retained for disposition by a veterinarian" or "condemned" (Barbut 2016). If the carcass is deemed condemnable, it may be reprocessed but must be disposed of if it is diseased following the strict guideline.

Periodically Check of Viability of Pathogenic Bacterial Cells in Chilling Environment Colonization of *Salmonella* and *Campylobacter* in the chicken gut or on the skin provides the chance for cross-contamination during poultry processing. Within the chicken's intestinal tract, these pathogenic bacterial loads can reach up to 10^8–10^9 CFU/ml (Barbut 2016). Fortunately, most carcasses leave processing plants with very minimum number of *Campylobacter* and/or *Salmonella* on meat or carcasses after chilling (Berrang et al. 2007). This number is in general less than the infectious dose of 500 bacterial cells of *Campylobacter* but above the infectious dose of *Salmonella* though the infectious dose of some serovars of *Salmonella* is higher than *Campylobacter* (Barbut 2016). However, if the viscera are not removed intact or properly or the skin is not cleaned properly after defeathering, it is possible to lead to fecal contamination of the carcasses providing the pathogens with an opportunity to colonize (Sutmoller 1997). *Salmonella* is a limited heat-resistant bacterium that is not fully inhibited by chilling but ceases to multiply at chilling temperatures. Even the carcasses or meat contains limited number of metabolically inactive *Salmonella* but that should not underscore the importance of cooking of poultry to an appropriate internal temperature, at least 70 °C, for killing all bacteria in and on the product (Barbut 2016). Prior to 2014, it was required in all poultry processing plants to chill the carcasses at 4.4 °C for 4–8 h depending on their weight (FSIS 2014) and that duration of chilling time and temperature were enough to cease *Campylobacter* growth as *Campylobacter* cannot multiply below 30 °C, but they can be converted to viable but non-culture cell (Barbut 2016). In August 2014, USDA introduced new guidelines for the inspection and chilling of poultry post-slaughter and made the information available in the website of the USDA Food Safety and Inspection Service (FSIS 2014). The newly developed guideline ensures that poultry processing plants must incorporate a chilling method in their individual hazard analysis and critical control point plans (FSIS 2014) and each plant must ensure that immediately after slaughter the carcasses are chilled to a temperature that prevents pathogens specifically *Salmonella* and *Campylobacter* proliferation (FSIS 2014).

Safer Storage After Packaging Creating appropriate environmental conditions of broiler products during storage and continuous monitoring of the facility will assist in the prevention of bacterial growth and control invasion of pest. Improper storage temperature or facility can result in microbial proliferation and cross-contamination

leading to food spoilage and/or poultry-associated foodborne illness. Temperature is the first of several environmental conditions that must be strictly regulated and monitored regularly. Post-processed poultry carcasses or products are safest stored by either chilling temperature or freezing condition. Chilled poultry carcasses or products must ensure that meat temperature is reduced and reached to 4 °C within 4 h of slaughter (Dave and Ghaly 2011), which will facilitate a shelf life of 2–3 weeks of the products (NRC 1988). In addition, most of the insect activity can be inhibited by creating the temperature below 4 °C, though some pests can survive long exposure to these temperatures (Brennan and Grandison 2012). Even low temperature is critical to control pathogenic bacterial growth and cross-contamination, but chilling temperature is used for chilling fresh poultry products that must be above freezing temperatures to ensure the quality of the products. Low-temperature controlling practices slow the growth of bacterial pathogens but do not inhibit completely. Freezing is a method that allows products to be stored longer, for example, storage temperature between −28.9 and − 40 °C can allow to store for months to a year. The maximum recommended storage temperature for frozen poultry is −23.3 °C (Barbut 2016). Researchers also found that freezing methods, particularly below −20 °C, are good in vitamin retention in poultry products. In some cases, nutrient levels have been remained well in frozen products than fresh products, though it depends upon the age of fresh product (NRC 1988). Storage or processing temperatures for poultry products between 4.4 and 60 °C are referred to as the "Danger Zone," and that results in rapid bacterial growth and spoilage of the products. Broiler products stored above 4.4 °C for longer than 2 h should not be made available for consumers (Barbut 2016).

Water activity of poultry meat and percentage of humidity in the storage facility are also important factors for the storage of poultry products. Water activity is measured at the point when the relative humidity of air is in equilibrium with the humidity of the food product. Higher water content or moist products such as fresh cuts of poultry can have a water activity of 0.85 or above. To freeze the poultry products properly, higher water activity levels are recommended. While higher moisture is necessary to maintain superior quality of frozen poultry products, at the same time, higher water content in chicken meat also increases the potential risk for several pathogenic bacterial growth specifically *Campylobacter*, *Salmonella*, and *Staphylococcus* because minimum water activity levels of 0.98, 0.94, and 0.86, respectively, are required for the growth and multiplication of these bacterial pathogens. Since the minimum humidity for the growth of some pathogenic bacteria can overlap with the optimum humidity for poultry storage, chilling with antimicrobial components or another barrier is necessary to control microorganisms (Dave and Ghaly 2011).

Methods and materials used for packaging of poultry products are other important steps for controlling the storage environment. Proper packaging serves to protect products from cross-contamination or floor contamination, delay spoilage through limiting growth of microbe or contamination, and regulate gaseous conditions or air exchange during storage. Barbut (2016) recommended that modified atmospheric packaging is one of the processes of controlling the mixture of atmospheric gases within the packaging to minimize microbial growth. Researcher also found that higher levels of carbon dioxide are effective in reducing bacterial growth

on poultry products (Brennan and Grandison 2012). Consequently, it is important that packaging has a good barrier for oxygen and/or carbon dioxide to prevent any gas from migrating (Barbut 2016). Overall, leak-proof packaging such as plastics showed significant influence on poultry product storage and is effective at minimizing contamination of poultry products with spoilage microorganisms (Food Safety Authority of Ireland 2011). Though plastics are a commonly accepted packaging material due to durability, barrier permeability, and resistance to breakage, considering the environmental damage, more sustainable and environmentally friendly packaging materials are needed to introduce. All packaging materials should be sterilized prior to use for increasing the safety of storage (i.e., hydrogen peroxide) or pretreated with active ingredients (i.e., antioxidants, oxygen scavengers) to protect them throughout storage (Barbut 2016).

Proper Transportation and Handling Transportation of processed food products, specifically meat and animal products, is a crucial step in modern food transportation and marketing. Now, very often raw materials such as animal carcasses and other ingredients are all transported within the country as well as globally by land, sea, and air, and in many cases, products are needed to be in the carrier from as range few hours to few weeks (USDA-ERS 2015). In recent time, consumers all over the world vastly depend on imported foods, particularly frozen meat and other protein sources, or indirectly raw material used for food production, and retailers are required to display their products year-round. As such, imported poultry products from other countries are often integrated with local products and transported in the same carriers. Thus long-distance transport of many foods has become common, and many retailers vastly depend on it (Grandison 2012). Therefore, transportation of food is also considered a short-term storage, and control of temperature and humidity of the carrier, cleaning or sanitation, and limiting the cross-contamination of the product during transportation are important steps in food safety. In addition, training of vehicle drivers and loading and unloading workers and their basic knowledge in food safety and storage are also essential. In addition, improper transport causes physical and mechanical damages of food products and rapid changes in temperature and humidity, which may impose a high risk of compromising the product quality during transport are also needed to monitored. It is also important to make sure the products are delivered on time, and unscheduled delays need to be checked thoroughly.

6.5 Possible Mistakes at Consumer Level

Immediately after poultry products leave the supermarket, the potential for cross-contamination can increase, and the existing bacterial load on products can rise due to mishandling of products. Therefore, it is the consumer's responsibility to safely handle and prepare food for consumption. Without practicing proper hygiene and carefully handling food products, pathogens can potentially spread and increase risk

of illness. As human carelessness is one of the major causes of food contamination, mistakes or cross-contamination of the food products can be avoided with appropriate risk communication with and training of workers. Educating consumers concerning food hygiene has the potential to dramatically decrease foodborne illness.

Safe Transport Risk of contamination initiates as soon as a consumer begins to transport poultry packages from the grocery store. Within the grocery store, packages of poultry are usually stored on refrigerated shelves and are often slightly moist to touch. Any packaging that is moist or wet could possibly hold poultry juices that have leaked out to a certain extent. Consumer hands could become contaminated upon contact with these juices, which can remain contaminated even after they dry. This makes it easy for bacterial spread to other grocery items, posing a risk for illness when shoppers later consume contaminated food items. Produce products are high-risk items because they are often not cooked or sanitized prior to consumption. This creates ideal conditions for a food-poisoning incident to occur. This method of cross-contamination also creates a risk for other consumers and grocery store employees through conveyer belt contamination. When picking up poultry packages, keep it away from other food items in the grocery cart, and sanitize the hands immediately following contact. When bagging grocery items, placing the poultry in a separate plastic bag is also important. Maintaining awareness of this cross-contamination can minimize the chances of foodborne illnesses (Satin 2008).

Safe Storage There are several common mistakes that can be made by consumers when it comes to safe storage of poultry products. When raw poultry is purchased from the grocery store, it is important to immediately refrigerate the product temperature no greater than 39.9 °F. It should remain in the refrigerator for no longer than 1–2 days. Since refrigeration does not prevent growth of psychrotropic bacteria, extended storage of raw poultry at this temperature can result in bacterial proliferation and spoilage. Poultry stored for 2 days or longer should be kept in a freezer that maintains a temperature of −17.8 °C. Freezing poultry meat allows for storage up to a year. If for any reason the meat is left out on the counter for longer than 2 h, it should be disposed of immediately. In addition to temperature, proper packaging material is important in keeping poultry fresh as well as preventing freezer burn. Poultry products can be stored in the original packaging or repackaged. If a consumer chooses to repackage frozen chicken, it is essential to store the meat in airtight freezer containers or bags.

When purchasing a ready-to-eat prepared chicken, storage methods should be slightly different. Rotisserie chickens or restaurant-prepared leftovers can be refrigerated for 3–4 days in shallow storage containers. It is also safe to freeze this meat, but for best quality, it should be consumed within 4 months, under which condition, reusing of packaging materials or newly used boxes or containers may have some impact on meat quality.

Thawing Frozen poultry meat and poultry products are required to thaw prior to cooking. A common misconception is that raw chicken can be thawed on countertop at room temperature. This quick method of thawing chicken is hazardous and should

never be used. There are three alternative methods to safely thaw chicken. If possible, plan your meals ahead of time, and thaw the chicken in the refrigerator for 1–2 days. The meat can remain safe in the refrigerator for an additional day or two after thawing but should be refrozen if it is not prepared for any longer.

Poultry products may also be thawed in cold water. If this method is selected, the water must be changed every 30 min until thawed to ensure that the temperature remains cold. Poultry should be thawed in a leak-proof packaging to prevent bacteria from the environment from being introduced into the food. Cold-water thawing will take about an hour or less for a pound of boneless chicken breasts. For a 3–4 pound package of meat, thawing may take approximately 2–3 h. When thawing anything larger than 4 pounds, it is safest to thaw for 30 min per pound. With cold-water thawing, the meat must be cooked before refreezing to ensure safe consumption. While it may seem quicker and easier to thaw the chicken in hot water, this is not considered a safe method of thawing as it supports an environment for bacterial growth.

Microwave thawing can provide a rapid method of preparing frozen poultry for cooking. If this method is selected, consumers must plan to cook the meat immediately after thawing. Certain parts of the meat may begin to cook during microwave thawing, which may bring the food into the "danger zone." Food should not remain at this temperature for any period of time, and it must be cooked before refreezing when using microwave thawing. If a consumer feels that there is absolutely no time to thaw the poultry, it is also safe to cook completely frozen meat. If this is the case, the food should be cooked for approximately 50% longer than the standard cooking time (USDA 2013a, b, c).

Food Preparation When handling raw poultry, it is critical to be cautious of cross-contamination. Preparing chicken involves the use of many different kitchen utensils, making it easy for clean kitchen supplies to come in contact with contaminated items. To avoid making this mistake, wash hands, countertops, cutting boards, and any utensils with soap and hot water immediately following contact with raw poultry meat. One of the easiest ways to transmit bacteria is through the dirt beneath the fingernails, so it is important to be thorough when scrubbing hands after contamination. When simultaneously preparing other products such as produce or side dishes, make sure to have a separate set of plates, cutting boards, and utensils from those used for chicken. Raw meat should never come in contact with cooked meat or any ready-to-eat food products. Other kitchen items that can harbor foodborne pathogens include dishtowels, sponges, and potholders. Not washing or replacing these items after contact with raw poultry is a hazardous mistake and increases likelihood of the spread of foodborne illness.

Other food preparation mistakes can arise from rinsing and marinating poultry. Prior to cooking, some consumers are in the habit of rinsing or soaking the raw poultry. This is not recommended because it increases the likelihood of cross-contamination through splashing of raw chicken juices onto countertops and other food products. Rinsing chicken will not help to eradicate bacteria. Marinating chicken is safe, but any leftover marinade must either be discarded or boiled prior to use. Reusing contaminated marinade on ready-to-eat chicken creates a high-risk scenario for foodborne illness (USDA 2015a, b).

Preparing a stuffed chicken or turkey, as consumers may do for the holidays, requires even more caution. Once a chicken is stuffed, it should be immediately placed in the oven at a temperature of at least 325 °F. It should be cooked until the stuffing reaches a safe temperature of 165 °F. The cooked poultry and stuffing should sit out for no longer than 2 h before refrigeration. It is important to never microwave a stuffed chicken. Due to rapid temperature increase while cooking, it is not likely that the stuffing will reach a temperature that will destroy any foodborne bacteria (USDA 2013b).

Cooking Cooking meat at high temperatures is the only way to eliminate pathogens from raw products to the point of safe consumption. A dangerous misconception is the idea that chicken can be cooked "medium" or "medium rare." Poultry is only safe to consume if it is cooked all the way through, which can be determined by observing color changes and measuring internal temperatures. Safely cooked poultry can be shades of white, tan, or even pink. While pink coloration is most often associated with raw meat, it is possible for cooked poultry to have a pink tinge as a result of the chemical changes during cooking. The red coloration of the meat comes from a protein called myoglobin that is fixed in the tissues. When myoglobin is combined with oxygen, it forms oxymyoglobin, producing a bright red color. The rest of the color comes from hemoglobin, a protein that occurs mostly in circulation. It is important to ensure that the inside of the cut meat no longer has the glossy appearance of raw poultry. If a consumer is unsure whether their poultry is cooked thoroughly through just observation, a food thermometer can be used to monitor the internal temperature of the meat. The thermometer should be placed in the innermost part of the breast, thigh, or wing. Even if the meat remains pink, it is safe to eat once it has reached 165 °F (USDA 2013a).

Depending on the cut of meat, thickness, and cooking method, the time to temperature ratio will vary. If the cooking equipment is functioning properly and the recipe instructions are followed accurately, the risk of pathogen survival is negligible. However, complications can arise when using microwave cooking because the meat is heated to its final temperature more rapidly. When this happens, the time portion of time to temperature relationship is not attained. In this case, the meat should rest in the oven for the remaining time called for once it has reached its final temperature (Satin 2008).

Handling of Leftovers Oftentimes, when cooking at home or eating out at a restaurant, consumers will have leftovers that they choose to save for later consumption. It is just as important to safely handle poultry products after they have been cooked and served. After the hot food is removed from the oven and leftovers are set aside, they should be promptly placed in the refrigerator. Some common mistake consumers make is leaving leftovers out at room temperature to cool down before refrigeration or freezing. It is important to cool food rapidly to the safe refrigeration temperature of 39.9 °F. When dealing with large portions of food, such as soup or a whole chicken, rapid cooling is best achieved when divided into smaller portions. Divide the food into small, shallow containers to accelerate the cooling process. For whole chickens or turkeys, cut items into smaller slices. Larger portions take a longer time to cool, permitting rapid multiplication of foodborne pathogens and increased risk of foodborne illness.

Leftovers stored in the refrigerator still have the potential to accumulate bacteria if not stored properly. It is important to consider the proper methods of wrapping poultry products for storage. Leftovers need to be wrapped in airtight packaging or sealed in storage containers. Appropriate storage will aid in the deterrence of bacterial pathogens, prevent odors from other foods stored nearby, and assist with moisture retention. Leftovers can remain refrigerated for up to 3–4 days and should then be transferred to the freezer for storage up to 3–4 months. For safety concerns, leftovers should remain safe indefinitely but will likely loose flavor and moisture when they are kept frozen for over 4 months (USDA 2015a).

6.6 Conclusion

The microbial safety of poultry products vastly depends on the farming and post-harvest level processes that occur after the bird is harvested from the farm, which include slaughtering/beheading, chilling, defeathering, processing, dressing, packing, and properly storage at the appropriate temperatures and safer transportation from the storage to the retail stores or restaurants. To control the growth of microbes both pathogenic and nonpathogenic (spoilage microbes), frozen products must be maintained in a frozen state from the processing facility to the consumer house or restaurants. Quality of water used in pre- and post-harvest levels, specifically noting the presence of coliforms/microbial contamination, must be ensured and monitored during recycling. Quality assurance/control must be developed in a written form following the FSIS guideline, thoroughly identifying possible critical food safety contamination points and Standard Sanitation Operating Procedures.

References

Barbut, S. (2016). *The science of poultry and meat processing*. http://download.poultryandmeatprocessing.com/v01/SciPoultryAndMeatProcessing%20-%20Barbut%20-%2015%20Microbilogy%20%26%20Sanitation%20-%20v01.pdf

Berrang, M. E., Bailey, J. S., Altekruse, S. F., Patel, B., Shaw, W. K., Meinersmann, R. J., et al. (2007). Prevalence and numbers of *Campylobacter* on broiler carcasses collected at rehang and post-chill in 20 US processing plants. *Journal of Food Protection, 70*(7), 1556–1560.

Brant, A. W. (1974). The current status of poultry chilling in Europe. *Poultry Science, 53*, 1291–1295.

Brennan, J. G., & Grandison, A. S. (2012). Postharvest handling and preparation of foods for processing. In *Food processing handbook* (pp. 10–11). Weinheim, Germany: Wiley-VCH Verlag.

CDC (Center for Disease Control and Prevention). (2013). *Food Safety progress report*. http://www.cdc.gov/foodnet/data/trends/trends-2013-progress.html

Dave, D., & Ghaly, A. E. (2011). Meat spoilage mechanisms and preservation techniques: A critical review. *American Journal of Agricultural and Biological Sciences, 6*(4), 486–510.

Food Safety Authority of Ireland. (2011). *Recommendations for a practical control programme for Campylobacter in the poultry production and slaughter chain*. https://www.fsai.ie/recommendationsforapracticalcontrolprogrammeforcampylobacterinthepoultryproductionandslaughterchain.html

FSIS (Food Safety and Inspection Service). (2014). *Modernization of poultry slaughter inspection: Amendments to chilling requirements.* https://www.fsis.usda.gov/wps/wcm/connect/7a0a728e-3b29-49e9-9c1bec55f2f04887/Chilling-Requirements-1014.pdf?MOD=AJPERES

Grandison, A. S. (2012). Postharvest handling and preparation of foods for processing. In J. G. Brennan & A. S. Grandison (Eds.), *Food processing handbook* (2nd ed.). Weinheim, Germany: Wiley-VCH Verlag GmbH & Co.

Hafez, H. M. (1999). Poultry meat and food safety: Pre– and post-harvest approaches to reduce foodborne pathogens. *World's Poultry Science Journal, 55*(03), 269–280.

Hinton, M. H., Allen, V. M., Tinker, D. B., Gibson, C., & Wathes, C. M. (1996). The dispersal of bacteria during the defeathering of poultry. In M. H. Hinton & C. Rowlings (Eds.), *Factors affecting the microbial quality of meat. 2. Slaughter and dressing* (pp. 113–121). Bristol, UK: University of Bristol Press.

Hinton, M. H., & Corry, J. (1998). The decontamination of carcass meat. In B. Nagy & R. W. A. W. Mulder (Eds.), *Poultry and food safety* (pp. 23–31). Bristol, UK: European Communities.

Jacobs, L., Delezie, E., Duchateau, L., Goethals, K., & Tuyttens, F. A. (2017). Broiler chickens dead on arrival: Associated risk factors and welfare indicators. *Poultry Science, 96*(2), 259–265.

James, C., Vincent, C., de Andrade Lima, T. I., & James, S. J. (2006). The primary chilling of poultry carcasses - A review. *International Journal of Refrigeration, 29*, 847862.

Li, Y., & Slavik, M.F. (1996). Electric pasteurization method for reduction or elimination of bacteria on poultry carcasses and in processing water. In *Proceedings of Food Safety Consortium Annual Meeting*, Kansas City (pp. 58–67). (cited by White et al., 1997).

Marangoni, F., Corsello, G., Cricell, C., Ferrara, N., Ghiselli, A., Lucchin, L., et al. (2015). Role of poultry meat in a balanced diet aimed at maintaining health and wellbeing: An Italian consensus document. *Food & Nutrition Research, 59*, 27606.

Mead, G. C., Allen, V. M., Burton, C. H., & Corry, J. E. (2000). Microbial cross contamination during air chilling of poultry. *British Poultry Science, 41*, 158–162.

Mead, G., Lammerding, A. M., Cox, N., Doyle, M. P., Humbert, F., Kulikovskiy, A., et al. (2010). Scientific and technical factors affecting the setting of *Salmonella* criteria for raw poultry: A global perspective. *Journal of Food Protection, 73*(8), 1566–1590.

Nayak, R., Kenney, P. B., & Bissonnette, G. K. (2001). Inhibition and reversal of Salmonella typhimurium attachment to poultry skin using zinc chloride. *Journal of Food Protection, 64*, 456–461.

NRC (National Research Council). (1988). Committee on technological options to improve the nutritional attributes of animal products. In *Designing foods animal Product options in the marketplace*. Washington, DC: National Academy.

Nunes, F. (2011). *Scalding and plucking for optimum carcass quality and yield. Poultry World*. http://www.poultryworld.net/Broilers/Processing/2011/4/Scalding-and-plucking-for-optimum-carcass-quality-and-yield-WP008731W/

Pearson, A. D., Greenwood, M. H., Feltham, R. K., Healing, T. D., Donaldson, J., Jones, D. M., et al. (1996). Microbial ecology of Campylobacter jejuni in a United Kingdom chicken supply chain: Intermittent common source, vertical transmission, and amplification by flock propagation. *Applied Environmental Microbiology, 62*, 4614–4620.

Satin, M. (2008). *Sources of food-borne disease. Food alert: The ultimate sourcebook for food safety* (2nd ed.pp. 53–55). New York: Facts Page.

Scott, R. (2013). *Water reuse in poultry processing: Now addressed in the HACCP program (C 901)*. Athens, GA: The University of Georgia Cooperative Extension.

Singh, P., Lee, H. C., Chin, K. B., Ha, S. D., & Kang, I. (2015). Quantification of loosely associated and tightly associated bacteria on broiler carcass skin using swabbing, stomaching, and grinding methods. *Poultry Science, 94*, 3034–3039.

Slavik, M. F., Kim, J. W., & Walker, J. T. (1995). Reduction of *Salmonella* and *Campylobacter* on chicken carcasses by changing scalding temperature. *Journal of Food Protection, 58*(6), 689–691. https://doi.org/10.4315/0362-028X-58.6.689

Stahl, P. (1996). Slaughter and dressing of poultry. In M. H. Hinton & C. Rowlings (Eds.), *Factors affecting the microbial qualify of meat. 2. Slaughter and dressing* (pp. 99–106). Bristol, UK: University of Bristol Press.

Sutmoller, P. (1997). Contaminated food of animal origin: Hazards and risk management. *OIE Scientific and Technical Review, 16*(2), 1–27. http://siteresources.worldbank.org/INTARD/843432111114986030 0/20434404/ContaminatedFood.pdf

Thomson, J. E., Whitehead, W. K., & Mercuri, A. J. (1974). Chilling poultry meat-a literature review. *Poultry Science, 53*, 1268–1281.

Tinker, D. B., Gibson, C., Hinton, M. H., Wathes, C. M., & Allen, V. M. (1996). Defeathering-engineering developments. In M. H. Hinton & C. Rowlings (Eds.), *Factors affecting the microbial quality of meat. 2. Slaughter and dressing* (pp. 123–131). Bristol, UK: University of Bristol Press.

USDA (United States Department of Agriculture). (2013a). *The color of meat and poultry*. https://www.fsis.usda.gov/wps/wcm/connect/e8dad81f-f7fc-4574-893e-bae20cf8b215/Color_of_Meat_and_Poultry.pdf?MOD=AJPERES

USDA (United States Department of Agriculture). (2013b) *Stuffing and food safety*. https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/poultry-preparation/stuffing-and-food-safety

USDA (United States Department of Agriculture). (2013c). *The big thaw*. https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/safe-food-handling/the-big-thaw-safe-defrosting-methods-for-consumers/CT_Index

USDA (United States Department of Agriculture). (2015a). *Leftovers and food safety*. https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/safe-food-handling/leftovers-and-food-safety/ct_index

USDA (United States Department of Agriculture). (2015b). *Chicken from farm to table*. https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/poultry-preparation/chicken-from-farm-to-table/ct_index

USDA-ERS (United States Department of Agriculture-Economic Research Service). (2015). *Organic agriculture*. http://www.ers.usda.gov/data-products.aspx

Van der Wal, P. G., & Muller, R. W. A. W. (1996). Modern scalding technology for pigs and poultry. In M. H. Hinton & C. Rowlings (Eds.), *Factors affecting the microbial quality of meat. 2. Slaughter and dressing* (pp. 107–111). Bristol, UK: University of Bristol Press.

Veerkamp, C. (1999). Challenges for water management in processing. *Poultry Process Worldwide, 7*, 20.

Yang, H., Li, Y., & Johnson, M. G. (2001). Survival and death of *Salmonella* Typhimurium and *Campylobacter jejuni* in processing water and on chicken skin during poultry scalding and chilling. *Journal of Food Protection, 64*(6), 770–776.

Chapter 7
Advances in Packaging of Poultry Meat Products

Sunil Mangalassary

7.1 Introduction

Packaging is a socio-scientific discipline that operates to ensure the consumer that goods are delivered in the best condition intended for their use (Lockhart 1997). Although product protection inside the package is the primary function of packaging, it extends beyond to facilitate many other functions such as containment, convenience, communication, and marketing. Packaging and related traits influence purchase intentions and decisions by consumers (McMillan 2017). The protection function includes the protection of food from outside environmental effects, physical damage, physicochemical deterioration, microbial spoilage, and contamination by foodborne pathogens. Packaging is one of the final steps in food manufacturing and processing and therefore considered as one of the final hurdles in enhancing the microbiological safety of the food product. Many of the emerging active packaging technologies focus on enhancing the microbiological safety of food products. Also, recent innovations make food packaging a suitable component for the hurdle technology concept of food preservation.

Meat, poultry, and seafood are in the category of highly perishable foods, and along with proper processing and storage, packaging plays a significant role in enhancing the safety and quality of these products. Meat and some of the meat products support bacterial growth and therefore susceptible to spoilage and possess safety concerns. With the demand from retailers to extend the shelf life in a cost-effective manner and to meet the consumer expectations in relation to convenience and quality, the food packaging industry has been continuously coming with appropriate innovations (Kerry et al. 2006). Packaging of meat has always been a

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challenge as many properties of the product, especially in the fresh stage, will be affected by the type of polymer used and the internal environment within the package. Some of those properties include microbial quality, lipid stability, color, water holding capacity, and tenderness (Chen et al. 2015; McMillan 2017). The factors that need to be considered in the packaging of processed meat products are dehydration, lipid oxidation, discoloration, and the loss of flavor (Mondry 1996). The packaging options vary with the type of meat (species), fresh or processed, type of storage, and display.

Consumption of poultry meat has been on the increase universally for the last several years due to various reasons. Worldwide, poultry grew rapidly and surpassed pork as the preferred animal protein in 2016 (OECD-FAO 2017). The major poultry meat quality attributes are appearance, texture, juiciness, and flavor (Fletcher 2002). Poultry meat and products contain higher amounts of unsaturated fatty acids that are susceptible to lipid oxidation which is a major concern due to its deleterious effects on flavor, color, texture, and nutrients. The microbiological safety and quality of poultry meat and products are important as contamination with foodborne pathogens remains an important public health issue (Mead 2004) along with the loss resulting from microbial spoilage. Various packaging systems, the type of packaging polymer used, and the gaseous atmosphere inside the package are some of the important packaging-related factors that can influence the abovementioned qualities of poultry meat and products. Therefore, selection of the packaging system including the type of polymers is an important consideration in maintaining the quality especially the microbiological quality and safety of the products. This chapter will discuss the advancements in poultry packaging with respect to enhancing the poultry meat and product quality with a focus on microbiological quality.

7.2 Packaging and Its Effect on Microbiological Quality of Poultry Meat and Products

Poultry meat can be contaminated with many types of microorganisms, including those producing spoilage during refrigerated storage and foodborne pathogens. The bacteria on broiler meat immediately after processing reportedly include *Micrococcus*, gram-positive rods, *Flavobacterium*, *Enterobacteriaceae*, *Psuedomonas,* and *Acinetobacter* (Charles et al. 2006). The primary population of bacteria reported on spoiled refrigerated poultry meat is psychrotrophic organisms, predominantly *Psuedomonas* spp. (Russel et al. 1996). Although *Salmonella* and *Campylobacter* spp. are the predominant foodborne pathogens associated with poultry, other bacteria such as *Clostridium perfringens*, *Escherichia coli* O 157: H7, and *Listeria monocytogenes* (especially in ready to eat (RTE) poultry meat products) are also indicated in foodborne disease outbreaks resulting from the consumption of poultry meat and products (Corry and Atbay 2001).

Packaging influences the microbiological quality of the contained product both in direct and indirect ways. The primary function of a package is to protect the product, including prevention of recontamination of poultry meat and products from spoilage and pathogenic bacteria. Controlling physical damages such as bruises also limit the enhanced bacterial growth within the product. Modern packaging techniques such as modified atmosphere packaging (MAP) and vacuum packaging help to control the gaseous environment within the package, thereby restricting the growth of certain type of microorganisms (Blacha et al. 2014; Meredith et al. 2014). The use of oxygen scavengers mostly in the form of sachets inside the primary package is also an innovative method, which can exert some antibacterial effect against aerobic bacteria (Cichello 2015; Demirhan and Candogan 2017). Antimicrobial packaging, one of the most researched forms of active packaging, is a direct packaging method specifically designed to reduce or inhibit specific or general bacterial populations present on the food product (Mulla et al. 2017; Olaimat and Holley 2015).

7.3 Packaging Systems for Poultry Meat and Products

The packaging materials and system designed for poultry must ensure excellent presentation and extended shelf life. The shelf life of the packaged product is related to the packaging functions such as protection against physical (bruises) chemical (oxidation) changes and microbial (spoilage and pathogenic) contamination. Appearance is the primary characteristic consumers consider to select a food product, especially packaged fresh meat. The packaging system and polymer used should be able to maintain product quality throughout the supply chain and during the intended storage period.

There are different packaging options for raw chilled and processed meats to maintain the desired properties during storage and display, which in turn depend on the consumer expectations (McMillan 2017). The microbiological and other qualities are related to the primary packaging that protects the food and the one which consumers are familiar with. The most commonly used primary packaging for poultry meat is a polymer film wrap or overwrap or a composite layer containing paper, foil, and cellophane (Dawson 2010). Some of the current methods used for fresh poultry include preformed trays wrapped using flexible packaging film for whole birds, flexible packaging films wrapped around the whole bird meat without a tray, portioned poultry placed on preformed trays using flexible plastic film as a complete wrap or tray seal, and portioned poultry wrapped in a flexible film without a tray (ULMA 2014). In case of ready to eat (RTE) poultry meat products, in addition to plastic films and trays, paper bags and folding cartons with windowed portions are generally used. The tray and overwrap packaging format is still the most widely used method for fresh poultry packaging (Dawson 2010). Mostly, whole poultry in trays is packed under MAP technology using stretch and barrier films. For frozen and fresh products, packaging with shrink stretch film is a common practice. For frozen and fresh meat tray-less packages, vacuum packaging is also an option commonly used.

7.4 Modified Atmosphere Packaging (MAP)

Modified atmosphere packaging (MAP) and related packaging technologies are increasingly used to extend the shelf life of various food products. The use of MAP to preserve meat and poultry products has been extensively studied, and significant improvements in shelf life extensions have been observed compared to chilled storage under normal air in the package head space. The MAP is defined as the removal and/or replacement of the atmosphere surrounding the product before sealing mostly using vapor barrier polymers (McMillan et al. 1999). The MAP includes vacuum packaging, which removes most of the air before the product is sealed in a barrier polymer, and controlled atmosphere packaging (CAP) where after modification of the internal atmosphere, a continuous monitoring and control are employed to maintain a stable gas atmosphere, temperature, and humidity within the package throughout the storage (Brody 1989; Phillips 1996). Another modification of vacuum packaging, vacuum skin packaging, involves placing the product on a tray and wrapping it in a film under a vacuum at higher temperature, where the heat causes softening of the top film, which then tightly covers the product (Kameník et al. 2014). Modified atmosphere packaging for meat requires a barrier of both moisture and gas permeation, which is achieved through selecting appropriate packaging polymers with aforementioned barrier properties (McMillan 2008).

7.4.1 Gases Used in MAP

The basic concept of MAP is the replacement of air surrounding the product by a mixture of atmospheric gases different in proportion from that present in air (Rao and Sachindra 2002).

Modified atmosphere packaging mostly uses three gases, nitrogen (N_2), carbon dioxide (CO_2), and oxygen (O_2), in different proportions depending on the product and desired shelf life and quality characteristics. The use of carbon monoxide (CO) has been recently employed in MAP essentially to prolong the red color of meat, especially beef (Luno et al. 2000; Van Rooyen et al. 2017). A few studies have investigated the use of CO on quality parameters in poultry meat (Fraqueza and Barreto 2011; Kudra et al. 2012). Argon, (an inert, odorless, and tasteless gas) as an alternative to N_2, has been allowed to be used for MAP in the European Union (Herbert et al. 2013).

Nitrogen is an inert gas with no antimicrobial activity, and its primary function is to act as a filler when significant amount of oxygen is removed (Meredith et al. 2014). When used along with CO_2, N_2 prevents package collapse resulting from CO_2 absorption into the meat.

Carbon dioxide plays a major role in contributing to the antimicrobial function of MAP, and the use of CO_2-enriched atmosphere inside food packages to extend shelf life has been well established. The effectiveness of MAP is generally determined

by the amount of CO_2 available to dissolve into the food (Gill 1996). The possible mechanisms of antimicrobial activity include formation of carbonic acid within the cell after permeating into the cell, thereby decreasing the intracellular pH and activities (Wolfe 1980), specific inhibition of decarboxylating enzymes (King and Nagel 1975), non-specific inhibition of susceptible non-decarboxylating enzymes (Ranson et al. 1960), and alteration of membrane properties inhibiting the membrane functions (Sears and Eisenberg 1961). In an earlier work by Hotchkiss et al. (1985), it was demonstrated that chicken quarters packaged in glass jars containing 80% CO_2 and 20% O_2 showed significant reduction in aerobic count and the predominant species shifted from gram-negative to gram-positive. The sensory evaluation of meat also yielded higher scores for CO_2-stored samples compared to the ones stored in air. The posttreatment or residual effect of CO_2 is an important factor because a continuous contact of the gas with meat is required for antimicrobial effect, which is often achieved only by using greater than 80% CO_2 in the gas mixture (Rao and Sachindra 2002). A study by Rodriguez et al. (2014) determined the shelf life of RTE cooked chicken fillets stored in atmospheres that were modified with different concentrations of CO_2 and studied the relationship between gas concentration and bacterial growth. The treatments used were aerobic, vacuum, and 10, 30, 50, 70, and 90% CO_2 with the remaining volume filled with N_2. The increased package CO_2 concentration caused a reduction in the growth rate of various bacteria, and treatment with 90% CO_2 appeared promising as a method to increase product shelf life. Another technique called soluble gas stabilization (SGS), where a sufficient amount of CO_2 can be dissolved into the product before retail packaging, has the potential to prevent package collapse without compromising package quality (Sivertsvik and Jensen 2005). The suitability of SGS to dissolve CO_2 into skinless chicken breast fillets before MAP was investigated by Rotbakk et al. (2006). An increased SGS time of 12 h instead of 2 h before MAP increased CO_2 content in the packaged fillets and prevented package collapse. The SGS treatment significantly decreased aerobic plate count, *Enterobacteriaceae* and *Psuedomonas* spp. counts, compared with no SGS treatment although all these counts increased over the storage period. Al-Nehlawi et al. (2013) investigated the effects of an aerobic MAP (70% CO_2, 15% O_2, and 15% N_2) with and without a CO_2 3 h SGS pre-treatment of chicken drumsticks. The greater availability of CO_2 in SGS samples resulted in lower counts of aerobic bacteria and *Psuedomonas* compared to normal MAP samples. Also the incidence of package collapse as a result of CO_2 absorption into meat was also significantly reduced in SGS samples.

Oxygen is a major factor influencing the shelf life of food products including meat. It is used in fresh meat packaging to maintain the red color through the formation of oxymyoglobin. But color is not as significant as a criterion for consumers in the purchase of poultry meat as that of beef (Millar et al. 1994). Additionally, a few studies have indicated that the color of the ground chicken and turkey were not found to be stable when packaged in atmospheres rich in O_2 (Saucier et al. 2000; Dhananjayan et al. 2006). Dhananjayan et al. (2006) demonstrated that the surface color of ground turkey breast patties in an 80% O_2 atmosphere is less stable compared with patties under 97% CO_2 atmosphere. The presence of O_2 in the internal

packaging environment can favor the growth of aerobic spoilage bacteria and cause oxidation of lipids. The composition of the microflora on the product changes depending on the concentration of O_2 used in MAP (Phillips 1996). Rossaint et al. (2015) compared the effect of atmospheres containing high O_2 (70% O_2 30% CO_2) vs. high N_2 (70% N_2 and 30% CO_2) on spoilage during refrigerated storage of poultry fillets. Anaerobic conditions favored the growth of *Lactobacilli* spp., whereas high O_2 atmosphere favored the growth of *Brochothrix thermosphacta*. There was no significant difference in total viable count (TVC) between the two treatments, and overall, the results suggest that high O_2 packaging had no additional beneficial effect on quality maintenance and shelf life of fresh poultry.

A few studies have investigated the effect of incorporating CO to the gas mixture used in the MAP of poultry meat. A study was conducted to evaluate the effect of anaerobic gas mixture with CO at a level of 0.5% along with different concentrations of CO_2 and N_2 on the growth of spoilage flora, color, and lipid oxidation stability of turkey meat under MAP stored at 0 °C (Fraqueza and Barreto 2011). The lipid oxidation was not prevented either by CO or CO_2. A reduction in spoilage flora, especially *B. thermosphacta*, was evident in gas mixtures containing high level of CO_2. The presence of CO was helpful in maintaining the bright pink color of turkey meat. Another study by Kudra et al. (2012) examined the effect of combining irradiation with MAP with high CO_2 and CO mixture against *Campylobacter jejuni* in chicken breast meat. Results revealed that the effect irradiation on the inhibition of the pathogen was significant than the MAP even with a high CO_2 level.

7.4.2 MAP: Enhancement of Microbiological Safety and Shelf Life

Raw meat and poultry are highly perishable food products, which readily support microbial growth even under refrigerated storage (Sade et al. 2013). MAP is gaining popularity as one of the most commonly used nonthermal methods for food preservation. It has been demonstrated that MAP significantly enhances the shelf life of various food products, including poultry products. The shelf life of poultry packaged in modified atmospheres depends on gas composition and concentration, storage temperature, the degree of initial contamination, film permeability to O_2 and CO_2, and combination with any other preservative technique.

In the USA, about 48 million foodborne illnesses are reported annually causing an estimated $77.7 billion annual loss to the US economy (CDC 2011; Scharrf 2012). *Salmonella* and *Campylobacter* are the leading bacterial causes of foodborne illnesses in the USA, and together these pathogens contribute 20% of the total foodborne illnesses (Scallan et al. 2011). One of the major food sources of these two pathogens is poultry. *L. monocytogenes* is yet another major foodborne pathogen contaminating RTE poultry products (Murphy et al. 2003; Mangalassary et al. 2007).

Poultry meat is often contaminated with *C. jejuni* during poultry processing, and human campylobacteriosis is most frequently associated with the consumption of contaminated poultry products (Skarp et al. 2016). The microaerophilic and capnophilic nature of *C. jejuni* demands attention when employing MAP to control its growth. It requires both O_2 and CO_2 for growth preferably at 5–10% and 1–10%, respectively (Bolton and Coates 1983). Oh et al. (2017) reported the prevalence of certain strains of hyper-aerotolerant strains of *C. jejuni* in poultry meat and studied their survival under different gas combinations under laboratory experimental conditions. They reported that a high concentration of CO_2 (>97%) reduced the viability of aerotolerant *C. jejuni* both in culture media and poultry meat. Meredith et al. (2014) investigated the effect of different MAP gaseous combinations on *Campylobacter* on poultry fillets and observed that the optimum concentration to reduce *Campylobacter* and extend shelf life was 40:30:30 CO_2:O_2:N_2, which achieved a shelf life of more than 14 days.

The prevalence of *Salmonella* in poultry products has always been a concern for the industry and regulatory agencies. Additional control measures are always being sought to reduce the prevalence of this pathogen in poultry products. The use of MAP often along with the combination of other preservative technologies has been found to be only moderately or not effective in controlling *Salmonella* on poultry products (Hulánková et al. 2010; Kudra et al. 2011; Nair et al. 2015). Hulánková et al. (2010) conducted a study to determine whether low numbers of *Salmonella* Enteritidis in the presence of natural microflora would survive on chicken legs stored in 30% CO_2/70% N_2 and 20% CO_2/80% O_2. Contrary to their hypothesis, even low numbers of the pathogen survived well on the surface of the poultry at 3 °C in both modified atmospheres tested. When a gas mixture of 99.5% CO_2 and 0.5% CO was used in combination with irradiation to study the survival of *S.* Typhimurium on chicken breast, it was observed that MAP did not exert any significant inhibitory effect on the pathogen compared to irradiation (Kudra et al. 2011). A recent study by Nair et al. (2015) investigated the effect of combination of a natural antimicrobial agent, carvacrol, with a MAP gaseous mixture of 95% CO_2 and 5% O_2 and found that the treatments resulted up to 2 log cfu/g reduction in *Salmonella* (*S.* Heidelberg, *S.* Typhimurium, *S.* Enteritidis) on turkey breast cutlets during a 7-day storage at 4 °C.

L. monocytogenes is a major threat to the food industry as a post-processing contaminant and commonly indicated in RTE poultry products. The ability of this organism to grow under refrigeration temperature makes it a significant public health threat. The growth behavior of *L. monocytogenes* in the presence of natural flora on poultry breast fillets under oxygen-rich and nitrogen-rich atmospheres (70% O_2/30% CO_2 and 70% N_2/30% CO_2) was studied by Herbert and Kreyenschmidt (2015). There was no significant increase in *L. monocytogenes* population during 20 days of storage in the O_2-enriched atmosphere as compared to a 3 log cfu/g increase in 70% N_2/30% CO_2 atmosphere. *B. thermosphacta* dominated the spoilage flora under O_2-enriched atmosphere and competitively suppressed the growth of *L. monocytogenes*.

7.5 Active Packaging Technologies

Active packaging is defined as an innovative packaging system or technologies that allow the product and its environment to interact to extend the shelf life and to ensure the microbial safety while maintaining other qualities of the packaged product (Ahvenainen 2003). These technologies modify the gas environment by removing gases from or adding gases to the package headspace. The internal atmosphere may be controlled by substances that absorb or release gases or vapors (Lopez-Rubio et al. 2004). Active packaging is typically found in two types of systems, sachets and pads, which are placed inside of packages, and active ingredients are incorporated directly into packaging materials. Various active technologies include antimicrobial and antioxidant packaging systems: oxygen, carbon dioxide, moisture, ethylene, and flavor absorbers; ethanol, carbon dioxide, and preservative emitters; self-heating, self-cooling packages; and UV- and surface-treated packages (Kerry et al. 2006).

7.5.1 Antimicrobial Packaging Systems

Antimicrobial packaging is a promising method of active packaging technology, and it can be used as one of the final hurdles in achieving the safety of a food product. In antimicrobial packaging, agents may be coated, incorporated, immobilized, or surface modified onto a packaging material. Various types of antimicrobial agents such as silver ions, sorbates, nitrites, organic acids, bacteriocins, and phytochemicals (from plant sources such as clove, cinnamon, thyme, oregano, thyme) have been researched for their efficacy after incorporating them into the polymer matrix through abovementioned techniques (Suppakul et al. 2003; Sung et al. 2013). The research conducted during the last several years to develop "green" packaging by incorporating bioactive antimicrobial compounds into bio-based polymers is a major step toward attaining sustainability in food packaging applications (Lopez-Rubio et al. 2006; Robertson 2014).

Most of the antimicrobial packaging systems that are currently developed require a close contact with the food product and the polymer for ensuring the diffusion of the antimicrobial compound from the polymer matrix to the surface of food except in a few systems, where volatile compounds with antimicrobial properties are used. With regard to poultry, there is a possibility of using both of the abovementioned systems as poultry fresh meat and products commonly use vacuum packaging and vacuum skin packaging where a direct contact is always established between the food product and polymer. In the same way, volatile compounds released from a sachet placed inside a package or release of volatile compounds from the overwrap in an aerobic or modified atmosphere packaging systems can be used for enhancing food safety.

A commonly studied group of antimicrobial compounds that have been found effective in poultry meat is essential oils. The antimicrobial activity of essential oils, including clove (eugenol), oregano (carvacrol), cinnamon (cinnamaldehyde), garlic (allicin), and mustard (isothiocyanate), has been established through many studies during the last several years (Irkin and Esmer 2015). Many of those compounds are considered generally recognized as safe (GRAS) by the Food and Drug Administration. Various food packaging materials that are used to incorporate essential oils include low-density polyethylene (LDPE), polypropylene, and chitosan (Ribeiro-Santos et al. 2017). A chemically modified linear low-density polyethylene (LLDPED) was coated with clove oil (0.5 g coated onto a 9 × 5 cm film), and its antimicrobial activity in chicken meat inoculated with *S*. Typhimurium and *L. monocytogenes* during a 21-day refrigerated storage was studied by Mulla et al. (2017). A complete inhibition of both pathogens was found on day 7 (5 log cfu/g for *L*. monocytogenes vs. 4 log cfu/g for *S*. Typhimurium), and no growth was detected after 21 days. The antimicrobial activity of clove oil has been attributed to its main functional component, eugenol. Lee et al. (2016) applied a fish (skate) gelatin film containing thyme oil to chicken tenderloins inoculated with *L. monocytogenes* and *E. coli* O157:H7 and observed that the antimicrobial film exerted a 2 log reduction in both bacterial counts after 10-day storage. Standardization of essential oils for their application in packaging is critical, and by combining with other preservative mechanisms, the desired antimicrobial effect can be obtained without producing undesirable changes in the flavor (Burt 2004).

Bacteriocins are another group of natural antimicrobial compounds that have been tested extensively for application in antimicrobial packaging. Bacteriocins are ribosomally synthesized polypeptides possessing bactericidal activity that are rapidly digested by proteases in the human digestive tract (Joerger et al. 2000). The most commonly used bacteriocins in food applications include nisin and pediocin produced by *Lactococcus lactis* and *Pediococcus acidilactici*, respectively. Nisin is a bacteriocin which was approved for use in food in 1969 and was awarded generally recognized as safe status in the USA in 1988 (FDA 1988). Nisin is effective in a number of food systems, inhibiting the growth of a wide range of gram-positive bacteria, including many important foodborne pathogens such as *L. monocytogenes* (Mangalassary et al. 2008; Matthews et al. 2010). Natarajan and Sheldon (2000) coated three types of polymer films (polyvinyl chloride (PVC), LDPE, and nylon) with nisin and applied to broiler drumstick skin samples inoculated with *S*. Typhimurium. The nisin-treated polymer films resulted up to a 2 log reduction in pathogen counts by 24 h. In addition, the researchers reported that shelf life was extended by 0.6–2.2 days following a 3-min immersion in a nisin-containing solution and subsequent storage in a foam tray pack containing nisin-treated PVC overwrap and nisin-treated absorbent tray pad. Similarly, when thermally compacted soy films were made with the incorporation of nisin and lauric acid and tested against *L. monocytogenes* inoculated onto a turkey bologna (Dawson et al. 2002), it was found that film containing both antimicrobials reduced the bacterial counts by 1 log after 21 days of storage.

Different biopolymers, such as proteins, lipids, and polysaccharides with the incorporation of various antimicrobial agents, have been found to be effective as edible coatings on various food products. Although these coatings do not perform all the functions of a typical packaging material, edible coatings can influence the quality of food product by controlling many factors such as permeability, food safety, and quality. Different types of edible films containing various antimicrobial agents have been studied in poultry meat and products (Fernández-Pan et al. 2014; Olaimat et al. 2014; Olaimat and Holley 2015). Whey protein isolate (WPI) edible coatings with oregano or clove essential oil were developed and applied onto chicken fillets (Fernández-Pan et al. 2014) to assess the effect on selected spoilage flora. Films with 20 g/kg of oregano essential oil showed their efficacy by doubling the storage time of chicken breast (from 6 to 13 days), keeping most of the microbiological groups below the recommended limits for distribution and consumption of chicken breast. Olaimat et al. (2014) evaluated the antimicrobial activity of an edible film (0.2% κ-carrageenan/2% chitosan-based coating) containing allyl isothiocyanate or deodorized oriental mustard extract against a 4 strain *C. jejuni* cocktail (6.2 $\log_{10}$ CFU/g) on vacuum-packaged fresh chicken breasts during 4 °C storage. κ-Carrageenan/chitosan-based coatings containing 50 or 100 μl/g allyl isothiocyanate reduced viable *C. jejuni* to undetectable levels on chicken breast after 5 days at 4 °C.

Emitting sachets and absorbent pads are one of the most successful applications of active food packaging, including antimicrobial packaging (Otoni et al. 2016). The sachets used are of two types: one where the antimicrobial compounds are generated in situ inside the sachets and released and sachets that carry and release antimicrobial compounds (Otoni et al. 2016). Some of the antimicrobial compounds that have been used in emitting sachets that produce them in situ include allyl isothiocyanate and chlorine dioxide (Ma 2012; Gómez-López et al. 2009). Soares et al. (2008) developed antimicrobial sachets by incorporating liquid allyl isothiocyanate into a porous high-density polyethylene resin as a carrier. The carrier polymer was then placed inside a non-woven fabric, which was heat sealed to form the antimicrobial sachets. A controlled released chlorine dioxide sachet applied with MAP was evaluated for its ability to control the growth of *S.* Typhimurium and *L. monocytogenes* on raw chicken breast during refrigerated storage (Shin et al. 2010). Inoculated fresh chicken samples with and without chlorine dioxide sachets were packaged in air or 30% CO_2/70% N_2 and stored at 4 °C for 21 days. The maximum microbial reduction in MAP with chlorine dioxide sachet was 0.68 log cfu/g for *S.* Typhimurium and 1.87 log cfu/g for *L. monocytogenes*. The exudate inside the packaged meat can enhance the microbial growth and negatively impact the sensory qualities of the product. In order to avoid this risk, absorbent pads have been used widely by the food industry (Otoni et al. 2016). A three-layer absorbent pad was prepared by alternating perforated polyethylene, cellulose, and polyethylene layers by Oral et al. (2009). This absorbent pad was sprayed with oregano oil and was intended to soak up chicken exudates for preventing microbial growth in the nutrient-rich exudates.

7.5.2 O_2 Scavengers

The presence of O_2 in a package can enhance lipid oxidation and resulting spoilage in meat products. It will also help the growth of aerobic microbes, odor and off-flavor development, and color and nutritional losses (Hogan and Kerry 2008). Since MAP or vacuum packaging often cannot remove the oxygen completely from a package, the use of active O_2 scavengers/absorbers in the form of labels or passive nanocomposites or incorporated in the polymer layers is necessary to absorb the necessary residual oxygen within the package (Ahmed et al. 2017). Oxygen scavenging systems used in meat products are commercially available in the form of sachets in the package, which include FreshMax® (Multisorb Technologies, Inc.) that is based on the oxidation of iron and OxyCatch™ (Kyodo Printing Company, Ltd.), where O_2 is trapped by cerium oxide particles (Ahmed et al. 2017). A polymer-based O_2 scavenger, Cryovac® OS2000 (Sealed Air Corporation, USA), is a multilayer flexible film that is activated by ionizing radiation (Speer et al. 2009). The effect of an O_2 absorber and a citrus extract (0.1 and 0.2 ml/100 g) on shelf life extension of ground chicken stored at 4 °C was investigated by Mexis et al. (2012). A product shelf life extension of 4–5 days using the combination of O_2 absorber and citrus extract as compared to an aerobically packaged control sample was obtained.

7.5.3 CO_2 Emitters

Carbon dioxide inhibits a wide range of aerobic bacteria causing increased lag phase and generation time during the logarithmic phase of microbial growth, thereby acting complementary to O_2 scavenging (Suppakul et al. 2003). Carbon dioxide emitters used in meat and poultry include CO_2® Fresh Pads (where drip loss from the product will be absorbed into the pad and reacts with citric acid and sodium carbonate present in the pad producing CO_2) and UltraZap® XtendaPak pads (Realini and Marcos 2014). Holck et al. (2014) investigated the shelf life of chicken fillets under different CO_2 concentrations at 4 °C and observed that storage in 100% CO_2 both with and without CO_2 emitter sachet gave an additional 7 days extension of shelf life compared to 60% CO_2. The storage in 100% CO_2 alone resulted in the collapse (due to the dissolving of the gas into the meat tissue) of the package resulting in significant drip loss, whereas the use of emitter sachet prevented the collapse and drip loss.

7.6 Hurdle Concept with Packaging Applications

Consumer demands for healthier foods that retain sensory and nutritional properties have persuaded the industry to find ways to minimize the adverse effects of preservation techniques thereby leading to the emergence of “hurdle” concept in food

preservation (Leistner 2000). The basic principle of hurdle technology centers around the use of multiple hurdles to attack various mechanisms of bacterial survival so that the intensities of individual technologies used in the combination can be minimized to preserve the sensory and nutritional qualities of food. Many types of packaging methods have been successfully employed as a component of hurdle technique in combination with various other preservation technologies. Various methods used along with packaging include irradiation, high-pressure processing, surface application of antimicrobials, and in-package pasteurization. The most frequently used packaging method in combination with other methods is MAP.

Modified atmosphere packaging has been studied extensively to evaluate the potential benefits in combination with other technologies. Packaging is a critical factor affecting the quality of irradiated meat. Modification of packaging methods can minimize the quality deterioration of irradiated meat (Nam et al. 2007). The combined effect of gamma irradiation (2 and 4 kGy) and MAP (30% CO_2/70% N_2 and 70% CO_2/30% N_2) on shelf life extension of fresh chicken meat stored under refrigeration was investigated (Chouliara et al. 2008). The combination of MAP (70% CO_2/30% N_2) and the higher irradiation dose of 4 kGy resulted in significant reduction of total viable count, *Pseudomonas* spp., lactic acid bacteria (LAB), yeasts, *Brochothrix thermosphacta*, and *Enterobacteriaceae*. However, on the contrary, Kudra et al. (2011, 2012) studied the combination of irradiation with MAP (CO_2 + CO) on chicken breast inoculated with *S*. Typhimurium and *C. jejuni* and found that MAP failed to exert a significant effect in reducing the pathogen count compared to irradiation. Jiménez et al. (1999) found that combining MAP (70% CO_2 30%N_2) with a 1% acetic acid pre-treatment decreased the counts of spoilage bacteria in chicken breast stored at 4 °C for 21 days. Similarly the combined effect of chitosan (dipping in 1 g/100 ml solution) and MAP (70% CO_2 and 30% N_2) on shelf life extension of chicken fillet was monitored for 14 days by Latou et al. (2014). The results indicated that the combination treatment resulted in a significant reduction in total bacterial count, LAB, *Psuedomonas*, and *Enterobacteriaceae* and maintained the product quality throughout the storage period. In another study, Petrou et al. (2012) determined the combined effect of chitosan (1.5% w/v) and oregano oil (0.25% v/w) application along with MAP (70% CO_2 30% N_2) on the shelf life of chicken breast during a 21-day refrigerated storage. The combined treatment resulted in a significant reduction of total aerobic count, LAB, *B. thermosphacta*, *Psuedomonas*, yeast, and mold along with maintaining the acceptable sensory characteristics.

High-pressure processing (HPP) is a novel preservation technology that results in significant enhancement in microbiological quality without compromising sensory qualities such as texture, color, and appearance. The contribution of MAP (50% CO_2 50% N_2) in enhancing the shelf life of high-pressure-treated raw poultry sausages was examined by Lerasle et al. (2014) during a 22-day storage. Interestingly, compared to HPP, MAP did not exert a significant effect on enhancing the microbiological quality although it limited lipid oxidation.

Antimicrobial polymers and edible coatings were also used along with other preservation methods to enhance shelf life of meat products. The inhibitory effects of in-package pasteurization combined with a nisin (7%, w/w) containing wheat gluten film were tested over an 8-week storage period against *L. monocytogenes* and

S. Typhimurium inoculated on refrigerated bologna (McCormick et al. 2003). Combining both treatments significantly reduced *L. monocytogenes* populations and prevented outgrowth over the 2-month storage period, but provided no added inhibitory effect against *S*. Typhimurium compared with only pasteurization. The effects of HPP in conjunction with coriander oil-based active packaging on the surface of RTE chicken breast were investigated as post-processing treatment to reduce *L. monocytogenes* (Stratakos et al. 2015). The combination of HPP and active packaging resulted in a synergistic effect reducing the pathogen counts to below the detection limit throughout the 60-day storage period at 4 °C. Likewise, Hassanzadeh et al. (2017) evaluated the combined effect of low-dose gamma irradiation (2.5 kGy) and chitosan edible coatings containing grape seed extract on the quality of chicken meat during 21 days of storage at 4 °C. Results indicated that irradiation and active coating significantly reduced aerobic mesophilic and psychrotrophic counts with at least a 14-day extension of shelf life.

7.7 Intelligent Packaging

Intelligent packaging is defined as a packaging system to detect, sense, and record any deterioration inside the food package to enhance food safety, improve quality, and warn about possible problems during food transport and storage (Yam et al. 2005). It actually fulfills the "communication" function of the packaging. Knowledge about the product quality, the packaging, and environment establishes a responsibility throughout the storage, transport, distribution, and sale (Fuertes et al. 2016). Intelligent packaging systems are packaging technologies that through indicators placed inside and outside of the package, monitor interaction between food, the packaging, and the environment (Biji et al. 2015). Two basic types of smart package devices are data carriers (bar code labels and RFID tags) that are used to transmit data and package indicators (time-temperature indicators, gas indicators, and biosensors) that are used to monitor the external environment and, whenever appropriate, issue warnings (Yam 2012). Many years of research and development have been invested in developing intelligent packaging systems which are often used to indicate various microbial and biochemical changes in food products (Brody 2014). Poultry meat being a highly perishable food and vulnerable to bacterial contamination, the application of intelligent packaging systems to ensure the safety of poultry meat and products is an encouraging step toward attaining safety and consumer satisfaction.

7.7.1 Indicators

Indicators are devices that give information on the presence or absence of a substance or the degree of interaction between two substances by changing characteristics such as color (Mohebi and Marquez 2015).

7.7.1.1 Time-Temperature Indicators

Temperature is one of the most important extrinsic factors that affect microbial growth, and variations in temperature during transport and storage can compromise the safety and shelf life of perishable food products, including meat, poultry, and fish. Time-temperature indicators (TTIs) are designed for continuous monitoring of time and temperature history of chilled and frozen products throughout the food chain (Lee and Rahman 2014). Time-temperature indicators available in the market are based on physical, chemical, enzymatic, or biological processes (Kerry et al. 2006). The various types of TTIs function based on different reactions and principles. Some of the products include 3M MonitorMark® (3M Company), FreshCheck® (Temptime Corp.), VITSAB® (VITSAB International AB), OnVu™ (Ciba and Freshpoint™), and Tempix® (Tempix AB), and they all indicate temperature fluctuations through color changes in certain dyes used as a result of specific chemical reactions at high temperatures (Realini and Marcos 2014). Brizio and Prentice (2014) evaluated the applicability of a photochromic TTI (OnVu™ TTI B1) to monitor the time-temperature history and shelf life of chilled boneless chicken meat. A microbiological analysis was carried out throughout the storage period to establish a correlation with the color change of the indicator. The results from this study showed that the indicators showed a discoloration similar to the rate of deterioration of meat offering a dynamic shelf life label. A prototype of LAB-based TTI was applied to a vacuum-packaged chicken breast meat by Park et al. (2013) and reported that the response of TTI which was measured as titratable acidity was correlated with coliform counts in the product.

7.7.1.2 Gas Indicators

After food packaging, the gas composition within a package can change as a result of the activity of the food, the permeability of the packaging materials or leaks, microbial spoilage, and environmental conditions (Yam et al. 2005). The gas indicators are usually printed or immobilized inside the package to have a direct contact with gases, and they indicate the presence of oxygen, CO_2, water vapor, ethanol, and hydrogen sulfide. Oxygen indicators are most commonly used for food packaging applications such as Ageless Eye® (Mitsubishi), which can be inserted inside the package and the indicator changes color from pink to blue when oxygen concentration is above 0.5%. It can also detect improper sealing of MAP packages (Fang et al. 2017).

7.7.1.3 Freshness Indicators

A freshness indicator in a packaging system shows product quality using microbial metabolites resulting from growth such as glucose, acetic or lactic acids, ethanol, volatile nitrogen compounds, biogenic amines, and carbon dioxide (Mohebi and Marquez 2015). Commercial applications of freshness indicators include Toxin

Guard® by Toxin Alert Inc. to monitor *Psuedomonas* growth and SensorQ™ by FQSI Inc., which senses spoilage in fresh meat and poultry products (O'Grady and Kerry 2008). Kuswandi et al. (2014) fabricated a novel sticker sensor based on methyl red to detect the freshness of broiler chicken cuts. The methyl red/cellulose membrane as a freshness sensor functions based on pH increase as the basic spoilage volatile amines produced gradually in the package headspace, and subsequently, the color of the sensor will change from red to yellow for spoilage indication, which was visible to the naked eye.

7.7.2 Sensors

A sensor can be defined as a device used to detect, locate, or quantify energy or matter giving a signal for the detection or measurement of a physical or chemical property to which the device responds (Kerry et al. 2006). The various types of sensors include biosensors, chemical sensors, and gas sensors. Biosensors are used to detect, record, and transmit information pertaining to biological reactions. A bioreceptor present in the sensor recognizes the target analyte, and a transducer converts biochemical signals into a quantifiable electronic response (Yam et al. 2005). Toxin Guard® (Toxin Alert, Canada) is a visual diagnostic system based on antibodies printed on polyethylene-based plastic packaging material, which detect targeted bacteria such as *Salmonella* sp., *Campylobacter* sp., *E. coli*., and *Listeria* sp. (Bodenhamer et al. 2004). Determination of indicator headspace gases is a way to assess the quality of a meat product and the integrity of packaging, which in turn can be achieved through the development of intelligent packaging incorporating gas sensor technology (Kerry et al. 2006). The chemical sensor or the receptor is a chemical selective coating capable of detecting the presence, activity, composition, and concentration of a particular chemical or gas through surface adsorption (Biji et al. 2015). Nano-based sensors can be used to detect pathogens, chemical contaminants, spoilage, and product tampering and track ingredients or products through the processing chain (Liu et al. 2007).

7.7.3 Radiofrequency Identification (RFID) Tags

Radiofrequency identification tags are electronic information-based systems that use radiofrequency electromagnetic fields to transfer data from a tag attached to an object for tracing and identifying the object automatically (Mohebi and Marquez 2015). Radiofrequency identification tags affixed to food can be used for monitoring temperature of perishable foods. Mountable, non-integrated, and no flexible sensor-based RFID with tags are available in the market to monitor the temperature, relative humidity, light exposure, pressure, and pH of products. These tags detect possible interruptions of cold chain which are harmful to food quality and safety (Vanderroost et al. 2014).

7.8 Conclusion

The primary goal of food packaging is to contain food in a cost-effective way that satisfies the requirements of the industry and consumers. In addition, modern innovative packaging technologies enable the food industry to communicate and maintain food safety and integrity, in addition to providing consumers the convenience and confidence. There is a worldwide increase in the consumption of poultry meat and its products in recent years, and maintaining the quality and microbiological safety of these products is a top priority for the industry and regulatory agencies. Many of the recent advancements in packaging have been effectively applied to poultry meat and products. The extensive use of technologies such as MAP and vacuum skin packaging and a promising start of applying techniques such as TTIs and various sensors in poultry packaging will lead to wholesome and safe products in the future.

References

Ahmed, I., Lin, H., Zou, L., Brody, A. L., Li, Z., Qazi, I. M., et al. (2017). A comprehensive review on the application of active packaging technologies to muscle foods. *Food Control, 82*, 163–178.

Ahvenainen, R. (2003). Active and intelligent packaging: An introduction. In R. Ahvenainen (Ed.), *Novel food packaging techniques* (pp. 5–21). Cambridge, UK: Woodhead Publishing Ltd.

Al-Nehlawi, A., Saldo, J., Vega, L. F., & Guri, S. (2013). Effect of high carbon dioxide packaging and soluble gas stabilization pre-treatment on the shelf life and quality of chicken drumsticks. *Meat Science, 94*, 1–8.

Biji, K. B., Ravishankar, C. N., Mohan, C. O., & Gopal, T. K. S. (2015). Smart packaging system for food applications: A review. *Journal of Food Science and Technology, 52*, 6125–6135.

Blacha, I., Krischeck, C., & Klein, G. (2014). Influence of modified atmosphere packaging on meat quality parameters on turkey breast muscles. *Journal of Food Protection, 77*, 127–132.

Bodenhamer, W. T., Jackowski, G., & Davies E. (2004). *Toxin alert: Surface binding of an immunoglobulin to a flexible polymer using a water soluble varnish matrix*. United States patent: 66992973.

Bolton, F. J., & Coates, D. (1983). A study of the oxygen and carbon dioxide requirements of thermophilic campylobacters. *Journal of Clinical Pathology, 36*, 829–834.

Brizio, A. P. D. R., & Prentice, C. (2014). Use of smart photochromic indicator for dynamic monitoring of the shelf life of chilled chicken based products. *Meat Science, 96*, 1219–1226.

Brody, A. L. (1989). Modified atmosphere/vacuum packaging of meat. In A. L. Brody (Ed.), *Controlled/modified atmosphere/vacuum packaging of foods* (pp. 17–37). Trumbull, CT: Food & Nutrition Press.

Brody, A. L. (2014). Signaling food spoilage. *Food Technology, 7*, 73–75.

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods: A review. *International Journal of Food Microbiology, 94*, 223–253.

Centers for Disease Control and Prevention (CDC). (2011). *CDC estimates of foodborne illness in the United States*. Retrieved August 20, 2017, from http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html

Charles, N., Williams, S. K., & Rodrick, G. E. (2006). Effects of packaging systems on the natural microflora and acceptability of chicken breast meat. *Poultry Science, 85*, 1798–1801.

Chen, L., Zhou, G., & Zhang, W. (2015). Effects of high oxygen packaging on tenderness and water holding capacity of pork through protein oxidation. *Food and Bioprocess Technology, 8*(11), 2287–2297.

Chouliara, E., Badeka, A., Savvaidis, L., & Kontominas, M. G. (2008). Combined effect of irradiation and modified atmosphere packaging on shelf life extension of chicken breast meat: Microbiological, chemical, and sensory changes. *European Food Research and Technology, 226*, 877–888.

Cichello, S. A. (2015). Oxygen absorbers in food preservation: A review. *Journal of Food Science and Technology, 52*, 1889–1885.

Corry, J. E. L., & Atbay, H. L. (2001). Poultry as a source of Campylobacter and related organisms. *Journal of Applied Microbiology, 90*, 96S–114S.

Dawson, P. L. (2010). Packaging. In C. M. Owens, C. Alvarado, & A. R. Sams (Eds.), *Poultry meat processing* (pp. 101–123). Boca Raton, FL: Taylor & Francis.

Dawson, P. L., Carl, G. D., Acton, J., & Han, I. Y. (2002). Effect of lauric acid and nisin-impregnated soy-based films on the growth of Listeria monocytogenes on turkey bologna. *Poultry Science, 81*, 721–726.

Demirhan, B., & Candogan, K. (2017). Active packaging of chicken meats with modified atmosphere including oxygen scavengers. *Poultry Science, 96*, 1394–1401.

Dhananjayan, R., Han, I. Y., Acton, J. C., & Dawson, P. L. (2006). Growth depth effects of bacteria in ground turkey meat patties subjected to high carbon dioxide or high oxygen atmospheres. *Poultry Science, 85*, 1821–1828.

Fang, Z., Zhao, Y., Warner, R. D., & Johnson, H. K. (2017). Active and intelligent packaging in meat industry. *Trends in Food Science & Technology, 61*, 60–71.

FDA. (1988). Nisin preparation: Affirmation of GRAS status as a direct human food ingredient. *Food Registration, 58*, 11247–11250.

Fernández-Pan, I., Carrión-Granda, X., & Maté, J. I. (2014). Antimicrobial efficiency of edible coatings on the preservation of chicken breast fillets. *Food Control, 36*, 69–75.

Fletcher, D. L. (2002). Poultry meat quality. *World's Poultry Science Journal, 58*, 131–145.

Fraqueza, M. J., & Barreto, A. S. (2011). Gas mixtures approach to improve turkey meat shelf life under modified atmosphere packaging: The effect of carbon monoxide. *Poultry Science, 90*, 2076–2084.

Fuertes, G., Soto, I., Carrasco, R., Vargas, M., Sabattin, J., & Lagos, C. (2016). Intelligent packaging systems: Sensors and nanosensors to monitor food quality and safety. *Journal of Sensors, 2016*, 1–8. https://doi.org/10.1155/2016/4046061

Gill, C. O. (1996). Extending the storage life of raw chilled meats. *Meat Science, 43*, 9–109.

Gómez-López, V. M., Rajkovic, A., Ragaert, P., Smigic, N., & Devlieghere, F. (2009). Chlorine dioxide for minimally processed produce preservation: A review. *Trends in Food Science & Technology, 20*, 17–26.

Hassanzadeh, P., Tajik, H., Rohani, S. M. R., Moradi, M., Hashemi, M., & Aliakbarlu, J. (2017). Effect of functional chitosan coating and gamma irradiation on the shelf-life of chicken meat during refrigerated storage. *Radiation Physics and Chemistry, 141*, 103–109.

Herbert, U., & Kreyenschmidt, J. (2015). Comparison of oxygen and nitrogen enriched atmospheres on the growth of Listeria monocytogenes inoculated on poultry breast fillets. *Journal of Food Safety, 35*, 533–543.

Herbert, U., Rossaint, S., Khanna, M., & Kreyenschmidt, J. (2013). Comparison of argon-based and nitrogen-based modified atmosphere packaging on bacterial growth and product quality of chicken breast fillets. *Poultry Science, 92*, 1348–1356.

Hogan, S. A., & Kerry, J. P. (2008). Smart packaging of meat and poultry products. In J. P. Kerry & P. Butler (Eds.), *Smart packaging technologies for fast moving consumer goods* (pp. 33–54). West Sussex, UK: John Wiley & Sons.

Holck, A. L., Pettersen, M. K., Moen, M. H., & Sorheim, O. (2014). Prolonged shelf life and reduced drip loss of chicken filets by the use of carbon dioxide emitters and modified atmosphere packaging. *Journal of Food Protection, 77*, 1133–1141.

Hotchkiss, J. S., Baker, R. C., & Qureshi, R. A. (1985). Elevated carbon dioxide atmospheres for packaging poultry II. Effect of chicken quarters and bulk packages. *Poultry Science, 64*, 333–340.

Hulánková, R., Bořilová, G., & Steinhauserová, I. (2010). Influence of modified atmospheric packaging on the survival of Salmonella Enteritidis PT 8 on the surface of chilled chicken legs. *Acta Veterinaria Brno, 79*, S127–S132.

Irkin, R., & Esmer, O. K. (2015). Novel food packaging systems with natural antimicrobial agents. *Journal of Food Science and Technology, 52*, 6095–6111.

Jiménez, S. M., Salsi, M. S., Tiburzi, M. C., Rafaghelli, R. C., & Pirovani, M. E. (1999). Combined use of acetic acid treatment and modified atmosphere packaging for extending the shelf-life of chilled chicken breast portions. *Journal of Applied Microbiology, 87*, 339–347.

Joerger, R. D., Hoover, D. G., Barefoot, S. F., Harmon, K. M., Grinstead, D. A., & Nettles-Cutter, C. G. (2000). Bacteriocins. In J. Lederberg (Ed.), *Encyclopedia of microbiology* (Vol. 1, 2nd ed., pp. 383–397). San Diego, CA: Academic Press, Inc.

Kameník, J., Saláková, A., Pavlík, Z., Borˇilová, G., Hulanková, R., & Steinhauserová, I. (2014). Vacuum skin packaging and its effect on selected properties of beef and pork meat. *European Food Research and Technology, 239*, 395–402.

Kerry, J. P., O'Grady, M. N., & Hogan, S. A. (2006). Past, current and potential utilization of active and intelligent packaging systems for meat and muscle-based products: A review. *Meat Science, 74*, 113–140.

King, D. A., & Nagel, C. W. (1975). Influence of carbon dioxide upon the metabolism of *Psuedomonas aeruginosa*. *Journal of Food Science, 40*, 362–366.

Kudra, L. L., Sebranek, J. G., Dickson, J. S., Mendonca, A. F., Zhang, Q., Jackson-Davis, A., et al. (2011). Control of *Salmonella* enterica Typhimurium in chicken breast meat by irradiation combined with modified atmosphere packaging. *Journal of Food Protection, 74*, 1833–1839.

Kudra, L. L., Sebranek, J. G., Dickson, J. S., Mendonca, A. F., Zhang, Q., Jackson-Davis, A., et al. (2012). Control of Campylobacter Jejuni in chicken breast meat by irradiation combined with modified atmosphere packaging including carbon monoxide. *Journal of Food Protection, 75*, 1728–1733.

Kuswandi, B., Jayus, J., Octaviana, R., Abdullah, A., & Heng, L. Y. (2014). A novel on-package sticker sensor for real time monitoring of broiler chicken cut freshness. *Packaging Technology and Science, 27*, 69–81.

Latou, E., Mexis, S. F., Badeka, A. V., Kontakos, S., & Kontominas, M. G. (2014). Combined effect of chitosan and modified atmosphere packaging for shelf life extension of chicken breast fillets. *LWT-Food Science and Technology, 55*, 263–268.

Lee, K., Lee, J., Yang, H., & Song, K. B. (2016). Production and characterization of skate skin gelatin films incorporated with thyme essential oil and their application in chicken tenderloin packaging. *International Journal of Food Science & Technology, 51*, 1465–1472.

Lee, S. J., & Rahman, A. T. M. M. (2014). Intelligent packaging for food products. In J. H. Han (Ed.), *Innovations in food packaging* (2nd ed., pp. 171–209). San Diego, CA: Academic Press.

Leistner, L. (2000). Basic aspects of food preservation by hurdle technology. *International Journal of Food Microbiology, 55*, 181–186.

Lerasle, M., Fedrighi, M., Simonin, H., Anthoine, V., Rezé, S., Chéret, R., et al. (2014). Combined use of modified atmosphere packaging and high pressure to extend the shelf-life of raw poultry sausage. *Innovative Food Science & Emerging Technologies, 23*, 54–60.

Liu, Y., Chakrabartty, S., & Alocilja, E. (2007). Fundamental building blocks for molecular bio-wire based forward error-correcting biosensors. *Nanotechnology, 18*, 1–6.

Lockhart, H. E. (1997). A paradigm for packaging. *Packaging Technology and Science, 10*, 237–252.

Lopez-Rubio, A., Almenar, E., Hernandez-Munoz, P., Lagaron, J. M., Catala, R., & Gavara, R. (2004). Overview of active polymer-based packaging technologies for food applications. *Food Reviews International, 20*, 357–387.

Lopez-Rubio, A., Gavara, R., & Lagaron, M. (2006). Bioactive packaging: Turing food into healthy food through biomaterials. *Trends in Food Science & Technology, 17*, 567–575.

Luno, M., Roncales, P., Djenane, D., & Beltran, J. A. (2000). Beef shelf life in low O2 and high CO_2 atmospheres containing different low CO concentrations. *Meat Science, 55*, 413–419.

Ma, J. (2012). *Allyl isothiocyanate derived from oriental mustard meal as a natural antimicrobial to inhibit the growth of molds on bread*. MS thesis, University of Guelph, Guelph, ON.

Mangalassary, S., Han, I. Y., Rieck, J., Acton, J., & Dawson, P. L. (2008). Effect of combining nisin and/or lysozyme with in-package pasteurization for control of Listeria monocytogenes in ready-to-eat turkey bologna during refrigerated storage. *Food Microbiology, 25*, 866–870.

Mangalassary, S., Han, I. Y., Rieck, J., Acton, J., Jiang, X., & Dawson, P. (2007). Effect of combining nisin and/or lysozyme with in-package pasteurization on thermal inactivation of Listeria moocytogenes in ready to eat turkey bologna. *Journal of Food Protection, 70*, 2503–2511.

Matthews, B., Mangalassary, S., Darby, D., & Cooksey, K. (2010). Effectiveness of Barrier film with a cellulose coating that crries nisin blends for the Inhibition of Listeria Monocytogenes. *Packaging Technology and Science, 23*, 267–273.

McCormick, K. E., Han, I. Y., Acton, J. C., Sheldon, B. W., & Dawson, P. L. (2003). In-package pasteurization combined with biocide-impregnated films to inhibit *Listeria monocytogenes* and *Salmonella* Typhimurium in turkey bologna. *Journal of Food Science, 70*, M52–M57.

McMillan, K. W. (2008). Where is MAP going? A review and future potential of modified atmosphere packaging of meat. *Meat Science, 80*, 43–65.

McMillan, K. W. (2017). Advancements in meat packaging. *Meat Science, 132*, 153–162.

McMillan, K. W., Huang, N. Y., Ho, C. P., & Smith, B. S. (1999). Quality and shelf-life of meat in case-ready modified atmosphere packaging. In X. L. Xiong, F. Shahidi, & C. T. Ho (Eds.), *Quality attributes in muscle foods* (pp. 73–93). New York: ACS Symposium Series, Plenum Publishing Corporation.

Mead, G. C. (2004). Microbiological quality of poultry meat: A review. *Brazilian Journal of Poultry Science, 6*, 135–142.

Meredith, H., Valdramisid, V., Rotabakk, V. T., Sivertsvik, M., & McDowell, D. (2014). Effect of different modified atmosphere packaging gaseous combinations on Campylobacter and the shelf-life of chilled poultry fillets. *Food Micrbiology, 44*, 196–203.

Mexis, F., Chouliara, E., & Kontominas, M. G. (2012). Shelf life extension of ground chicken meat using an oxygen absorber and citrus extract. *LWT - Food Science and Technology, 49*, 21–27.

Millar, S., Wilson, R., Moss, B. W., & Ledward, D. A. (1994). Oxymyoglobin formation in meat and poultry. *Meat Science, 36*, 397–406.

Mohebi, E., & Marquez, L. (2015). Intelligent packaging in meat industry: An overview of existing solutions. *Journal of Food Science and Technology, 52*, 3947–3964.

Mondry, H. (1996). Packaging systems for processed meat. In S. A. Taylor, M. Raimundo, F. Severini, & J. M. Smulders (Eds.), *Meat quality and meat packaging* (pp. 323–333). Utrecht, Netherlands: ECCEAMST.

Mulla, M., Ahmed, J., Al-Attar, H., Castro-Aguirre, E., Arfat, Y. A., & Auras, R. (2017). Antimicrobial efficacy of clove essential oil infused into chemically modified LLDPE film for chicken meat packaging. *Food Control, 73*, 663–671.

Murphy, R. Y., Duncan, L. K., Driscoll, K. H., Marcy, J. A., & Beard, B. L. (2003). Thermal inactivation of Listeria monocytogenes on ready-to-eat turkey meat products during post-cook in-package pasteurization with hot water. *Journal of Food Protection, 666*, 1618–1622.

Nair, D. V. T., Kiess, A., Nannapaneni, R., Schilling, W., & Sharma, C. S. (2015). The combined efficacy of carvacrol and modified atmosphere packaging on the survival of Salmonella, Campylobacter jejuni, and lactic acid bacteria on turkey breast cutlets. *Food Microbiology, 49*, 134–141.

Nam, K. C., Ko, K. Y., Min, B. R., Ismail, H., Lee, E. J., Cordray, J., et al. (2007). Effects of oleoresin-tocopherol combinations on lipid oxidation, off-odor, and color of irradiated raw and cooked pork patties. *Meat Science, 75*, 61–70.

Natarajan, N., & Sheldon, B. W. (2000). Efficacy of nisin coated polymer film to inactivate *Salmonella* Typhimurium on fresh broiler skin. *Journal of Food Protection, 63*, 1189–1196.

O'Grady, M. N., & Kerry, J. P. (2008). Smart packaging technology. In F. Toldra (Ed.), *Meat biotechnology* (pp. 425–451). New York: Springer.

OECD-FAO. (2017). *OCED-FAO agricultural outlook 2017–2026*. Retrieved August 20, 2017, from https://doi.org/10.1787/agr_outlook-2017-en

Oh, E., McMullen, L. M., Chui, L., & Jeon, B. (2017). Differential survival of hyper-aerotolerant Campylobacter jejuni under different gas conditions. *Frontiers in Microbiology, 8*, 954.

Olaimat, A. N., Fang, Y., & Holley, R. A. (2014). inhibition of Campylobacter jejuni on fresh chicken breasts by κ-carrageenan/chitosan-based coatings containing allyl isothiocyanate or deodorized oriental mustard extract. *International Journal of Food Microbiology, 187*, 77–82.

Olaimat, A. N., & Holley, R. A. (2015). Control of Salmonella on fresh chicken breasts by k-carrageenan/chitosan-based coatings containing allyl isothiocyanate or deodorized Oriental mustard extract plus EDTA. *Food Microbiology, 48*, 83–88.

Oral, N., Vatansever, L., Sezer, C., Aydin, B., Guven, A., Gulmez, M., et al. (2009). Effect of absorbent pad containing oregano essential oil on the shelf life extension of overwrap packed chicken drumsticks stored at four degree Celsius. *Poultry Science, 88*, 1459–1465.

Otoni, C. G., Espitia, P. J. P., Avena-Bustillos, R. J., & McHugh, T. H. (2016). Trends in antimicrobial food packaging systems: Emitting sachets and absorbent pads. *Food Research International, 83*, 60–73.

Park, H. R., Kim, Y. A., Jung, S. W., Kim, H. C., & Lee, S. J. (2013). Response of microbial time temperature indicator to quality indices of chicken breast meat during storage. *Food Science and Biotechnology, 22*(4), 1145–1152.

Petrou, S., Tsiraki, M., Giatrakou, V., & Savvaidis, I. N. (2012). Chitosan dipping or oregano oil treatments, singly or combined on modified atmosphere packaged chicken breast meat. *International Journal of Food Microbiology, 156*(3), 264–271.

Phillips, C. A. (1996). Review: Modified atmosphere packaging and its effect on the microbiological quality and safety of produce. *International Journal of Food Science and Technology, 31*, 463–479.

Ranson, S. L., Walker, D. A., & Clarke, I. D. (1960). Effect of carbon dioxide on mitochondrial enzymes from Ricinus. *The Biochemical Journal, 76*, 216–221.

Rao, D. N., & Sachindra, N. M. (2002). Modified atmosphere and vacuum packaging of meat and poultry products. *Food Review International, 18*, 263–293.

Realini, C. E., & Marcos, B. (2014). Active and intelligent packaging systems for a modern society. *Meat Science, 98*, 404–419.

Ribeiro-Santos, R., Andraide, M., Ramos de Melo, N., & Sanches-Silva, A. (2017). Use of essential oils in active food packaging: Recent advances and future trends. *Trends in Food Science & Technology, 61*, 132–140.

Robertson, G. (2014). Biobased but not biodegradable. *Food Technology, 6*, 61–70.

Rodriguez, M. B. R., Conte Junior, C. A., Carneiro, C. S., Franco, R. M., & Mano, S. B. (2014). The effect of carbon dioxide on the shelf life of ready to eat shredded chicken breast stored under refrigeration. *Poultry Science, 93*, 194–199.

Rossaint, S., Klausmann, S., & Kreyenschmidt, J. (2015). Effect of high oxygen and oxygen free modified atmosphere packaging on the spoilage process of poultry breast fillets. *Poultry Science, 94*, 96–103.

Rotbakk, B. T., Birkeland, S., & Jeskrud, W. K. (2006). Effect of modified atmosphere packaging and soluble gas stabilization on the shelf life of skinless chicken breast fillets. *Journal of Food Science, 71*, S124–S131.

Russel, S. M., Fletcher, D. L., & Cox, N. A. (1996). Spoilage bacteria of fresh broiler chicken meat. *Poultry Science, 75*, 2041–2047.

Sade, E., Murros, A., & Bjorkroth, J. (2013). Predominant enterobacteria on modified-atmosphere packaged meat and poultry. *Food Microbiology, 34*, 252–258.

Saucier, L., Gendron, C., & Gari'epy, C. (2000). Shelf life of ground poultry meat stored under modified atmosphere. *Poultry Science, 79*, 1851–1856.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. A., Roy, S. L., et al. (2011). Foodborne illness acquired in the United States – major pathogens. *Emerging Infectious Diseases, 17*, 7–15.

Scharrf, R. L. (2012). Economic burden from health losses due to foodborne illness in the United States. *Journal of Food Protection, 75*, 123–131.

Sears, D. F., & Eisenberg, R. M. (1961). A model representing physiological role of CO_2 at the cell membrane. *The Journal of General Physiology, 44*, 869–887.

Shin, J., Harte, B., Selke, S., & Lee, Y. (2010). Use of a controlled chlorine dioxide system in combination with modified atmosphere packaging to control the growth of pathogens. *Journal of Food Quality, 34*, 220–228.

Sivertsvik, M., & Jensen, J. S. (2005). Solubility and absorption rate of carbon dioxide into non-respiring foods. Part 3: Cooked meat products. *Journal of Food Engineering, 70*, 499–505.

Skarp, C. P., Hanninen, M. L., & Rautelin, H. I. (2016). Campylobacteriosis: The role of poultry meat. *Clinical Microbiology and Infection, 22*, 103–109.

Soares, N. F. F., Pires, A. C. S., & Camilloto, G. P. (2008). *Sachê antimicrobiano para uso em alimentos*. Brazilian Patent PI 0603881–6 A.

Speer, D. V., Edwards, F. B., Beckwith, S. W., Rivett, J., & Kennedy, T. D. (2009). *Method of triggering a film containing an oxygen scavenger*. (US 7504045 B2). In Google Patents. Retrieved August 21, 2017, from http://www.google.com.tr/patents/US7504045

Stratakos, A., Delgado-Pando, G., Linton, M., Patterson, M. F., & Koidis, A. (2015). Synergism between high-pressure processing and active packaging against Listeria monocytogenes in ready-to-eat chicken breast. *Innovative Food Science & Emerging Technologies, 27*, 41–47.

Sung, S. Y., Sin, L. T., Tee, T. T., Bee, S. T., Rahmat, A. R., Rahman, W. A. W. A., et al. (2013). Antimicrobial agents for food packaging applications. *Trends in Food Science & Technology, 33*, 110–123.

Suppakul, P., Miltz, J., Sonneveld, K., & Bigger, S. W. (2003). Active packaging technologies with an emphasis on antimicrobial packaging and its applications. *Journal of Food Science, 68*, 408–420.

ULMA. (2014). *Poultry packaging solutions*. Retrieved August 17, 2017, from http://www.harpak-ulma.com/packaging-solutions/fresh-food-packaging/poultry

Van Rooyen, L. A., Allen, P., & O'Connor, D. I. (2017). The application of carbon monoxide in meat packaging needs to be re-evaluated within the EU: An overview. *Meat Science, 132*, 179–188.

Vanderroost, M., Ragert, P., Devlieghere, F., & Meulenaer, B. D. (2014). Intelligent food packaging: The next generation. *Trends in Food Science & Technology, 39*, 47–62.

Wolfe, S. K. (1980). Use of CO and $C0_2$ enriched atmospheres for meats, fish, and produce. *Food Technology*, 34, 55–58.

Yam, K. L. (2012). In K. L. Yam & D. S. Lee (Eds.), *Intelligent packaging to enhance food safety and quality* (pp. 137–152). Cambridge, UK: Woodhead Publishing Ltd.

Yam, K. L., Takhistov, P. T., & Miltz, J. (2005). Intelligent packaging: Concepts and applications. *Journal of Food Science, 70*, R1–R10.

Chapter 8
Advances in Vaccines for Controlling Foodborne *Salmonella* spp. in Poultry

Michael H. Kogut and Elizabeth Santin

8.1 Introduction

Salmonellosis is a zoonotic disease caused by the gram-negative enteric bacterium *Salmonella*. They are not restricted to particular host species, and their epidemiology can therefore be complex. Most are able to colonize the alimentary tract of animals without production of disease; more than 2500 serotypes have been described, mostly belonging to the species *S. enterica*. Infections with broad-host-range serovars, such as *S. typhimurium* and *S. enteritidis*, result in asymptomatic carriers that play a major role in *Salmonella* propagation in poultry and hence in food contamination. Vaccination that is the most practical and effective method to control salmonellosis in poultry, especially in breeders and layers, is well documented for the decline of salmonellosis in poultry products in the United Kingdom (O'Brien 2013). *Salmonella* vaccines decrease public health risk by reducing bacterial intestinal colonization and organ invasion and decrease horizontal transmission by reducing fecal shedding and environmental bacterial contamination. Numerous recent reviews have thoroughly detailed the *Salmonella* vaccines past and present for broiler and layer chickens (Revolledo and Ferreira 2012; Desin et al. 2013; Aehle and Curtiss 2017; Wigley and Barrow 2017). This review will briefly summarize *Salmonella* vaccine development since 2013. To successfully colonize the gastrointestinal tract of chickens, nonhost-specific *Salmonella enterica* serovars must evade and/or subvert components of both the innate and acquired immune systems. To do so, the bacteria encounter and use intestinal macrophages as both a host cell locally and as a protective transport cell to the internal organs. By

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surviving in the macrophage, *Salmonella* has the ability to endure the antibacterial mechanisms of an immune cell, thus evading all other host defenses and persisting in the chicken. Further, a series of bacterial and host factors are involved in the asymptomatic persistent infection in chickens. Therefore, we will also discuss the activities occurring at the host: pathogen interface in the intestine that presents problems that are probably involved in developing a complete sterile vaccine-induced immunity against *Salmonella*. Specifically, this review will focus an emphasis on factors that should be considered in evaluating and making constructive advances in *Salmonella* vaccines/vaccination including (1) the influence of the microbiota and the effect of the tolerogenic gut environment on vaccine efficacy, (2) bacterial variability and heterogeneity of the host innate immune response, (3) the multitude of mechanisms that *Salmonella* use to evade host immune defenses, and (4) the effect of *Salmonella*'*s* ability to alter local host immunometabolic tissue phenotype.

8.2 Host Immunity to *Salmonella*

Intestinal colonization by *Salmonella* of young poultry induces intestinal infiltration of polymorphonuclear (PMN, heterophils in poultry) which confers a high level of resistance to both bacterial invasion by virulent *Salmonella* and the associated pathological effects, including gastroenteritis and localization to the reproductive tract (Pomeroy 1988; Conlan and North 1992; Porter and Holt 1992). Further evidence for the primary role of PMN in the innate host defenses against *Salmonella* infections are the following: (1) large numbers of bacteria have been observed with inflammatory PMNs at the site of salmonellae penetration of the intestine (Takeuchi and Sprinz 1967; Lin et al. 1987; Foster et al. 2003); (2) heteropenic chickens have a greater sensitivity to infections with salmonellae (Kogut et al. 1993, 1994b); and (3) in vitro studies have presented strong evidence that PMNs are able to kill salmonellae (Van Dissel et al. 1986; Roof and Kramer 1989; Coe et al. 1992; Stabler et al. 1993). The results from these studies were further proof that the PMN is an important cell in the host defenses against *Salmonella* infections. Recognition of *Salmonella* bacterial components, such as LPS and flagellin by toll-like receptors 4 and 5 (TLR4 and TLR5), respectively, and release of inflammatory mediators and antibacterial products by avian heterophils are hallmarks of the involvement of these cells in controlling infections (Kogut et al. 1993, 1994a, b, 1995). Accompanying the cellular influx is the upregulation in pro-inflammatory cytokine mRNA expression (IL-6, IL-1β, IL-12, IL-18) (Berndt et al. 2006; Berndt et al. 2007; Kogut and Arsenault 2015, 2017; Kogut et al. 2015b). However, this heterophil response does not have a significant protective response against the salmonellae bacteria that remain in the luminal side of the cecal epithelium. Interestingly, this inflammatory response is largely resolved by 3–4 days post-infection (Babu et al. 2006; Setta et al. 2012b; Withange et al. 2004, 2005a, b) characterized by the

reduction of pro-inflammatory cytokines mRNA transcription in the cecum to non-infected control levels, and yet *Salmonella* can persist in the intestine and be shed in the feces for several weeks (Withange et al. 2005a, b).

Following the initial heterophil response, macrophages migrate to the site of infection. Macrophages possess TLR ligands recognizing *Salmonella* MAMPs including LPS, flagellin, and CpG oligonucleotides (He et al. 2007; Zhang et al. 2008; Wigley 2014). Macrophages are also capable of producing the pro-inflammatory cytokines, IL-12 and IL-18, which play a fundamental role in stimulating the T_H1-acquired responses to *Salmonella* infection (Wigley et al. 2006; He et al. 2011).

However, a number of the infecting *Salmonella* are able to persist within macrophages by evading most of the antimicrobial mechanisms (McIntrye et al. 1967; Helaine et al. 2010; Wigley 2014). The survival of these macrophage intracellular bacteria plays a crucial role in the pathogenesis of salmonellosis in poultry and will be discussed further later in this chapter.

Although *Salmonella* can colonize the intestine for weeks, a Th1-mediated acquired response resulting in an increased expression of IFN-γ in the intestine and extra-intestinal tissues develops to clear the bacteria, but is independent of B cell involvement (Beal et al. 2004a, b; Wigley et al. 2005; Withange et al. 2005a, b; Beal et al. 2006; Berndt et al. 2007). The production of IFN-γ appears to be due to the influx of γδ-T lymphocytes into the intestine (Berndt and Methner 2001; Berndt et al. 2006). The γδ-T lymphocytes also exhibit a profound increased expression of the pro-inflammatory cytokines, IL-12 and IL-18.

Clearance from the intestine is a slow process with the bacteria persisting asymptomatically for up to 10 weeks or more following infection. Mechanistically, numerous groups have demonstrated that early cecal pro-inflammatory signals following initial infection with ST or SE were dramatically downregulated 2–4 days after infection that is linked with the development of an anti-inflammatory, Th2 response (Withange et al. 2005a, b; Johanns et al. 2010; Setta et al. 2012a, b; Chausse et al. 2014; Kogut and Arsenault 2015; Shanmugasundaram et al. 2015) to increased expression of IL-10 and TGF-β, which suggests that the end of the disease resistance and the start of a disease-tolerant state were being initiated. It would seem likely that regulation of inflammatory immune responses, presumably by regulatory T cells (Tregs), allows *Salmonella* to persist within the gut for a number of weeks without disease to the bird. Such a "tolerogenic" response would have little or no impact on the bird itself but has public health consequences in allowing persistence for several weeks, particularly given broiler chickens are typically slaughtered at around 5 weeks of age. Subsequently, we have found an expansion of the CD4+ CD25+ T cell (Treg) population in the cecum of *Salmonella*-infected chickens (Shanmugasundaram et al. 2015). Functionally, the cecal Tregs had increased suppressive activity for T effector cells and had a profound increase in IL-10 mRNA transcription. In the murine model of ST infection, the ability of the bacteria to persist or be cleared has been found to be dependent on the presence and function of Tregs (Johanns et al. 2010; Shanmugasundaram et al. 2015).

8.3 Vaccines

The host immune response to pathogens in the earliest stages of infection is a critical determinant of disease resistance and susceptibility. Broiler chickens are highly susceptible to *Salmonella* infection during their first days post-hatch due to the relative functional immaturity of the immune response of the young birds (Desmidt et al. 1997) that leads to persistent infections (Gast and Benson 1995; Van Immerseel et al. 2009; Kogut et al. 2015a, b). The birds can remain infected until slaughter, which leads to introduction of *Salmonella* in the slaughterhouse and food chain (Heyndrickx et al. 2002). Unfortunately, vaccination against nonhost-specific *Salmonella* serotypes has yielded variable success rates when determined by intestinal and extra-intestinal organ colonization by the bacteria with live, attenuated vaccines being more successful than killed vaccines. However, the issue of colonization and contamination of the environment with the live vaccine is an ongoing issue due to the recent HACCP rules.

The intestinal mucosal surface is the major portal of entry for *Salmonella*. Nonetheless, very little research has concentrated on the development of *Salmonella* vaccines that can elicit mucosal immune responses that would provide a more efficient and directed host defense against *Salmonella* at its infection site. Most advances understanding of mucosal immunity and *Salmonella* have occurred in mammalian models (Patel and McCormick 2014). However, as reviewed recently by Paul Barrow (2007), studies on the mechanisms of protective mucosal immunity against *Salmonella* and the discovery of safe and effective mucosal adjuvants have renewed interest in the development and use of mucosal vaccines for *Salmonella* in poultry.

Lastly, since prevention of *Salmonella* infection early post-hatch period is vitally important in the control of salmonellosis, the oral administration of an attenuated, live *Salmonella* vaccine to day-old chicks provides protection against reinfection with closely related *Salmonella* organisms by intestinal colonization-inhibition. Functionally, colonization-inhibition acts as an innate immune component of a live vaccine and has been advocated and found to be effective against homologous and heterologous serotypes (Barrow 2007; Bohez et al. 2008; Methner et al. 2011; Braukmann et al. 2016).

Very few studies with salmonella vaccines have been done in broilers, most having been done with layers birds (Table 8.1). Most of the studies used attenuated gene mutants that affected the virulence of the microorganism. In some of the studies (De Cort et al. 2013, 2015), the immune response was not evaluated because it was believed that the time after vaccination and challenge is too short to induce an efficient immune response but that live attenuated vaccine induced a colonization-inhibition mechanism that protected the birds against subsequent challenge. Colonization-inhibition phenomenon was first described by as primarily a microbial physiological process and that did not result in the development of adaptive immunity nor bacteriophage/bacteriocin activity responsible for the protection. Methner et al. (2011) found that the inhibition between different strains within the same

Table 8.1 Literature review from latest studies with *Salmonella* vaccines in chickens

Vaccine/route	Difference from wild type	Bacterial recovery after challenge	Immune response against the vaccine	Reference
Subunits of OmpC protein from *Salmonella typhimurium* SPF layers/ intramuscular	Experimental groups: V1, OmpC alone; V2, rOmpC þ FIA; V3, OMP; C, PBS. All vaccinated at 4 weeks and booster at 7 weeks of age	All vaccinated groups showed reduction in recover of wild *Salmonella typhimurium* challenge strain in muscle, gizzard, liver, heart, and fecal swab compared to non-vaccinated bird	Increase of IgY in rOmpC þ FIA- and OMP-vaccinated groups compared to non-vaccinated and increase of stimulation indices of lymphocytes in vaccinated group compared to non-vaccinated	Prejit et al. (2013)
Inactivated (ghost) *Salmonella enteritidis*/ intramuscular layers	Constructed ghost cassette containing a sense λpR and an antisense ParaBAD promoter system	Reduction recover of wild SE in the cecum and liver from immunized chickens compared to non-immunized	Ghost-vaccinated birds showed an increase of IgY and sIgA and γδ, CD4, and CD8 T cell in the spleen compared to non-vaccinated group	Jawale and Lee (2014)
Inactivated electron bean *Salmonella enteritidis*/ intramuscular white leghorns	An eBeam dose of 2.5 kGy (kilograys) was used to inactivate a high titer (10^8 CFU) of SE cells	eBeam-vaccinated group showed lower SE colonization in the ceca, liver, spleen, and ovaries compared to unvaccinated group Unvaccinated birds have significantly higher cecum log 5.32 compared to 1.46 in vaccinated group	Vaccinated groups showed higher IgG titers against SE than non-vaccinated birds	Jesudhasan et al. (2015)

(continued)

Table 8.1 (continued)

Vaccine/route	Difference from wild type	Bacterial recovery after challenge	Immune response against the vaccine	Reference
Inactivated (ghost) *Salmonella typhimurium*/ intramuscular layers	Constructed ghost cassette containing a sense λpR and an antisense ParaBAD promoter system	Vaccinated birds showed lower bacterial recover in the liver, spleen, and cecum at 7 days and in the spleen and cecum at 14 days compared to non-vaccinated birds	Vaccinated group showed higher IgG and sIgA, CD4 and CD8 spleen cell, and IL-2 and IFN-γ than non-vaccinated birds	Jawale and Lee (2016)
Live *Salmonella* Gallinarum mutant ΔloncpxR plus SG LTB-secretion strain/ oral Hy-line brown layers	LTB enhance the both mucosa and cellular response	Vaccinated birds showed reduction in mortality (10 compared to 70% in non-vaccinated) and gross lesion compared to non-vaccinated birds	Vaccinated birds show increased IgY and sIgA and higher stimulation indices in lymphocytes compared to non-vaccinated birds	Jeon et al. (2013)
Live *Salmonella enteritidis* ΔSPI1-lon-fliC/ oral	The mutant vaccine is attenuated from virulence and enables serologic differentiation from infected birds	The vaccinated birds showed reduction on recover of wild challenge strain at 4 and 14 days after challenge compared to unvaccinated birds	Vaccinated birds show no anti-flagellin antibody what allow the differentiation from infected birds	Matulova et al. (2013)
Live *Salmonella enteritidis* ΔhilAssrAfliG mutant strain/ oral gavage	Deletion of genes hilA (SPI-1 cecal colonization factor), ssrA (pathogenicity factor), and fliG (flagellar rotor protein)	Reduction recover of wild *Salmonella enteritidis* challenge 7, 21, and 42 in the cecum, at 7 in the spleen, and at 16 and 23 days at cloacal swabs	Not evaluated	De Cort et al. (2013)

(continued)

Table 8.1 (continued)

Vaccine/route	Difference from wild type	Bacterial recovery after challenge	Immune response against the vaccine	Reference
Live *Salmonella enteritidis* LTB-secretion strain	LTB enhance the both mucosa and cellular response	Reduction of excretion of wild *Salmonella enteritidis* challenge in the liver and cecum in vaccinated birds at 7 and 14 days compared to non-vaccinated birds	SE LTB-secretion strain-vaccinated birds showed higher IgY and sIgA and circulated CD4+, CD8+, and TCR T cells cell than non-vaccinated and challenged birds	Nandre and Lee (2014)
Live *Salmonella typhimurium* PBAD-mviN/ oral	Replace mviN for arabinose-inducible promoter (PBAD) which induces bacteria lysis inside the host cell. Reduce survival inside macrophage	6 weeks after challenge, there is reduction of cecum *Salmonella* recover from log 7 in non-vaccinated birds to log 2 in bird vaccinated with ST mutant	The ST mutant-vaccinated birds showed higher levels of IgY	Rubinelli et al. (2015)
Live *Salmonella enteritidis* ΔloncpxRcpdB Mutran/SPF birds	The genes deleted are related to attachment and invasion of pathogen	Only deletion of three genes at the same time reduces the pathogenesis. The efficiency of vaccine was observed by reduction of the mortality rate from 80 to 20% in non-vaccinated and vaccinate group, respectively	Higher IgY titer in serum from vaccinated birds compared to non-vaccinated	Si et al. (2015)
Live *Salmonella enteritidis* ΔhilAssrAfliG mutant strain/ spray and water	Deletion of genes hilA (SPI-1 cecal colonization factor), ssrA (pathogenicity factor), and fliG (flagellar rotor protein)	The spray route was efficient to reduce the recovery SE wild strain challenge from cecum of birds 7 days. The water route have lower efficiency	Not evaluated	De Cort et al. (2015)

(continued)

Table 8.1 (continued)

Vaccine/route	Difference from wild type	Bacterial recovery after challenge	Immune response against the vaccine	Reference
Live nonflagellated (*fliD*–) mutants of *S. enteritidis* 11 with and without the virulence plasmid (pSEV) white leghorn	Nonflagellated (*fliD*–) mutant SEΔ155 is the lack of its serovar-specific virulence plasmid	Less invasive in the spleen and liver than wild SE11. The SE mutant-vaccinated group reduced cecal colonization and cloacal shedding of the highly virulent *S. enteritidis*	Vaccinated birds showed lower titers against of anti-flagellin which could be used as marker for vaccinated and non-vaccinated birds	Imre et al. (2015)
Live *Salmonella* Senftenberg Δ*lon* Δ*cpxR* vaccine/oral and intramuscular layers	The deleted genes are associated with increase in exopolysaccharide production and reduction of the cell division, increased invasiveness for the mutant strain, and also acquired the additional biochemical property of melibiose	The SS mutant-vaccinated group showed lower positive samples and recovery of SS wild challenge than non-vaccinated from 14 days post challenge	Higher humoral IgY and sIgA titers and greater lymphocyte proliferation responses than inoculated with wild SS from 7 days to 3 weeks post-vaccination	Kamble and Lee (2016)
Live *Salmonella* Montevideo Δ*lon*Δ*cpxR* mutant/oral and intramuscular SPF birds	The deleted genes are associated with increase in exopolysaccharide production and reduction of the cell division, increased invasiveness for the mutant strain, and also acquired the additional biochemical property of melibiose	Intramuscular and oral vaccinations reduce the log of SM wild challenge strain recover until 5 days after challenge, but the intramuscular route shows lower recovery of wild SM compared to unvaccinated and oral-vaccinated birds at 15 days after challenge	sIgA was higher in birds vaccinated by intramuscular and oral route compared to non-vaccinated from 2 to 4 weeks, IgG was higher in intramuscular-vaccinated birds compared to non-vaccinated from 2 to 4 weeks, but it was higher in oral-vaccinated birds only at 4 weeks compared to unvaccinated. The lymphocyte stimulation indices were higher in vaccinated birds	Lalsiamthara and Lee (2016)

(continued)

Table 8.1 (continued)

Vaccine/route	Difference from wild type	Bacterial recovery after challenge	Immune response against the vaccine	Reference
Live *Salmonella typhimurium* (Poulvac® ST)	Mutant ST	The vaccinated birds reduce the recovery of *Salmonella* Heidelberg challenge at 28 days from log 4 (unvaccinated) to 1, but the reduction in prevalence was from 60% in vaccinated and 70% unvaccinated birds was	Not evaluated	Muniz et al. (2017)
Live *Salmonella typhimurium* Δ*metRmetD* and live *Salmonella typhimurium* PBAD-mviN/ oral		Vaccinated birds from two vaccines presented lower recovery of wild challenge strain; the means + SD were 4.71 ± 1.41 log CFU/g for *metRmetD*, 2.62 ± 0.8 log CFU/g for *PBAD-mviN*, and 6.49 ± 0.61 log CFU/g for the unvaccinated group	IgG binding specifically to *Salmonella* proteins in ELISA showed that *metRmetD* mutant had a mean titer of 7840 ± 1711, while the *PBAD-mviN* strain had a mean titer of 4520 ± 1544, and the unvaccinated group mean titer was 1700 ± 352.5	Rubinelli et al. (2017)

O-group did not exceed a value of 2 $\log_{10}$ units compared with control, but unfortunately this study did not evaluate the immune response. Further, we have observed (Hayashi and Santin, non-published data) an increase of mRNA expression of IL-10 in the cecum of broilers as well as a reduction in *S.* Heidelberg challenge in the cecum of vaccinated birds compared to non-vaccinated birds. We hypothesized that the increase in mRNA IL-10 expression in gut mucosa could be a "tolerogenic" response that allows the bacteria stay in the lumen mucosa. Thus, this could be further proof that colonization-inhibition is associated with the innate immune response as previously described (Barrow 2007; Van Immerseel et al. 2009).

8.4 Issues/Perspectives to Improve Vaccine Development for *Salmonella*

8.4.1 Microbiome and Intestinal Immunity

The role of the intestinal microbiota in influencing the development and maturation of the immune system is not in question (Hooper et al. 2012; Hand 2016). The microbiota plays a fundamental role on the induction, training, and function of the host immune system. In return, the immune system maintains the symbiotic relationship of the host with these highly diverse and evolving microbes. Under homeostatic conditions, this immunity-microbiota cooperation regulates the induction of protective responses to pathogens and the maintenance of pathways that maintain tolerance to innocuous antigens.

8.4.2 Intestinal Immunity: Host Defense and Tolerance Toward Intestinal Microbes

The intestinal immune system differs from the systemic immune system because it (1) defends against pathogens and entry of excessive intestinal microbes while *simultaneously* (2) maintains a state of immune tolerance to resident intestinal microbes.

8.4.3 Host Defense Mechanisms

Intestinal epithelial cells provide a physical barrier between the luminal microbes and the underlying intestinal tissues by producing a mucus layer and secreting antimicrobial proteins that limit bacterial exposure to the epithelial cells (Yu et al. 2012; Patel and McCormick 2014). The production of IgA provides additional protection from luminal microbiota. Innate microbial sensing by epithelial cells, dendritic cells, and macrophages is mediated through PRRs such as TLRs and NLRs. Activation of PRRs on innate cells induces various pathways that mediate microbial killing and activate acquired immune cells. Macrophages and dendritic cells present antigens to naïve CD4 T cell Peyer's patches where the cytokine environment modulates the differentiation of CD4 T cell subsets (Th1, Th2, Th17) (Setta et al. 2012a, b; Wick 2011; Patel and McCormick 2014).

8.4.4 Tolerance Mechanisms

The same defense mechanisms that limit microbial entry into intestinal tissues also play a role in tolerance. However, the activation of PRRs on unique populations of dendritic cells and macrophages, in contrast, presents antigen to T cells that lead to

differentiation of T regulatory cells which are regulated by IL-10, TGF-β, and retinoic acid (Johanns et al. 2010).

8.4.5 *Ignoring the Influence of the Microbiome on Vaccine Efficacy*

Unlike the host genome, which is rarely manipulated by xenobiotic intervention, the microbiome is readily changeable by diet, antibiotics, infections by pathogens, and other environmental insults (Baurhoo et al. 2009; Chee et al. 2010; Roberts et al. 2015). The plasticity of the microbiome has been implicated in numerous disease conditions, and unfavorable alteration of the commensal structure of the microbiota is referred to as dysbiosis. Dysbiosis is manifested by a reduction in the number of tolerogenic bacteria and an overgrowth of potentially pathogenic bacteria (pathobionts) that can penetrate the gut and resulting in the loss of tolerance and the generation of an immune response directed against commensal bacteria (Lan et al. 2005; Dinev 2009; Yu et al. 2012; Wideman et al. 2012; Hand et al. 2012; Brown et al. 2015).

Therefore, it appears obvious that since the microbiota influences the development of the immune response and vaccine efficacy requires stimulating an immune response, the gut microbiota can have an impact on a vaccine-induced immunity. Yet there have been only a single study in poultry to evaluate this, probably due to the complex nature of the studies (Park et al. 2017). However, with high-throughput technologies available in conjunction with systems biology technologies to study the gene networks (transcriptomics) and signaling pathways (kinomics/proteomics) activated by the interactome between microbiome- and vaccine-derived factors and host immune cells, we should begin to develop an understanding of the role of the microbiota and vaccine efficacy. There are a number of challenges that must be met before such experiments can be successful. For example, we have only recently been able to correlate specific bacterial taxa with immune (cytokine gene expression) function (Oakley and Kogut 2016). Whether there is an association between these specific bacteria and *Salmonella* vaccine efficacy needs to be determined. Further, what type of vaccine (live, attenuated, killed, subunit) can influence these members of the microbiota or the microbiota as a whole? Another factor that needs to be considered in an experimental design is that the microbiome is readily changeable by husbandry and other host- and environmental-dependent events that would affect the data. Lastly, one will eventually need to consider the influence that other members of the microbiome (viruses, fungi, protozoa) play in vaccine efficacy.

8.5 Bacterial Physiology Affects Host Innate Immune Responsiveness

Salmonella organisms colonize the intestinal tract and penetrate the mucosal epithelium in the cecum. Invasion of the epithelium results in the release of chemokines that leads to an influx of phagocytic cells (heterophils and macrophages) to the site

of infection (Van Immerseel et al. 2002; Wigley 2014). Both phagocytic cell types recognize MAMPs, such as flagellin and LPS, on the surface of the bacteria using PRRs like TLR5 and TLR4, respectively (Wigley 2014). Both cell types possess microbicidal activities that function to kill the bacteria (Setta et al. 2012a, b; He et al. 2013; Genovese et al. 2013). However, heterophils are better able to kill the salmonellae bacteria via oxidative stress, whereas the macrophages are more likely to become host cells to the bacteria (Burton et al. 2014). Heterophils are the first responders and function to prevent bacterial invasion beyond the intestinal epithelium (Genovese et al. 2013). Interestingly, macrophages follow the heterophil influx, but *Salmonella* are able to invade macrophages and survive within these cells where they can be disseminated to different extra-intestinal tissues, such as the liver and spleen (Barrow et al. 1997; Hensel 2000; Wigley 2014).

However, two recent studies have shown that the macrophage-*Salmonella* interaction is much more diverse and random action has been portrayed in the literature (Avraham et al. 2015; Jensen et al. 2016). Specifically, the disparity in the activity of factors produced by individual bacterial cells induces a heterologous macrophage immune response (Avraham et al. 2015; Jensen et al. 2016). Therefore, instead of the description of a homogeneous outcome, i.e., all the *Salmonella* either survive within macrophages or all are killed by macrophages, there is actually a heterogeneous outcome with the macrophages that is determined by the physiology of the bacteria. For example, it is well known that live, attenuated *Salmonella* vaccines induce a better protection in chickens than killed vaccines (Lillehoj et al. 2000). Jensen et al. (2016) provide compelling evidence that this is due to the live vaccine inducing a more effective antigen-presenting macrophage or dendritic cell activities to elicit a more functional protective immune response than the killed vaccine. These results demonstrate that the host cell can respond to the variability of the bacteria to "influence the downstream events that impact the subsequent immune response" generated by the infection (Jensen et al. 2016). Further, *Salmonella* are able to detect the transition from the extracellular environment of the intestinal lumen to the intravacuolar environment inside a host cell, thereby prompting a modulation of gene expression that activates a number of virulence strategies, including the ability to modify its surface components, such as LPS, and other membrane constituents once inside macrophages (Eriksson et al. 2003) and secretion of compounds to alter macrophage response (Galán and Collmer 1999).

In addition to variability in bacterial phenotype that is dependent upon the intra- or extracellular environment, the macrophage exhibits subpopulations of cells that respond differentially to the same MAMPs (Avraham et al. 2015). This functional heterogeneity combined with the variability of the salmonellae cells can dramatically impact the outcome of an infection and/or the development of a vaccine-induced protective sterile immunity (Avraham et al. 2015). For example, macrophages that contain *Salmonella* that are not actively growing (dormant but physiologically alive) display the M1 pro-inflammatory phenotype, but do not kill the bacteria suggesting that the non-growing phenotype is an evasion mechanism to avoid recognition by intracellular innate PRRs (Saliba et al. 2016). However, macrophages containing actively growing bacteria exhibit the M2 anti-inflammatory phenotype as seen pre-

viously (Eisele et al. 2013; Saliba et al. 2016). In this case, the growing bacteria phenotype induces a reprogramming of the macrophage polarization (Gordon 2005; Mosser and Edwards 2008; Gordon and Martinez 2010) to avoid the bactericidal activity of the host immune cell.

8.5.1 Salmonella *Evasion of Host Immune Responses*

Salmonella possess a set of important factors genetically determined in two type III secretion systems (TTSS-1 andTTSS-2) encoded on *Salmonella* Pathogenicity Islands (SPI), particularly SPI-1 and SPI-2. The SPI-1 is essential for colonization in gut (Dieye et al. 2009), while both SPI-1 and SPI-2 are required for colonization of systemic organs (Dieye et al. 2009; Rychlik et al. 2009). A successful *Salmonella* infection of the intestinal tract depends initially on the outcome of the bacteria's encounter with the macrophage followed by the ability to persistently colonize the intestine and extra-intestinal organs despite the presence of a functional immune surveillance system. *Salmonella*'s ability to survive, colonize, and persist is dependent upon a series of bacterial factors that leads to the asymptomatic infection. *In context of this chapter*, *the main question is what affect do these evasion mechanisms have on a vaccine-induced protective immune response*; i.e., *does a protective immunity override these evasion mechanisms upon a challenge infection*? The main issue being that the efficacy of a vaccine is evaluated by the level of intestinal and systemic colonization and performance after vaccination and experimental infection using the oral or parenteral route of administration. No attempt has been made to evaluate the ability of the challenge strains to evade the vaccine-induced immune response. Although it has been clearly shown that vaccination of chickens results in a quantitative reduced level and duration of intestinal colonization and reduced extra-intestinal organ invasion by *Salmonella* challenge organisms and reduced egg contamination following vaccination under experimental conditions (Gantois et al. 2006), a challenge infection with a wild-type strain will not result in absolute protection against intestinal colonization (Revolledo and Ferreira 2012).

8.5.2 Evasion of Intestinal Epithelial Cell Defense Systems

The first "phase" of a *Salmonella enterica* infection begins with the invasion of and penetration through the epithelium lining the intestinal tract (Chappell et al. 2009; Wigley 2014). *Salmonella* interacts with the epithelial cells by releasing two type III secretion systems that are programmed within its pathogenicity islands 1 (SPI-1) and 2 (SPI-2) (Waterman and Holden 2003; Galan and Wolf-Watz 2006; Ashida et al. 2012). The T3SS secretes protein effectors that exploit the host's cell biology to facilitate bacterial entry and intracellular survival and to modulate the host immune response. The SPI-1 facilitates entry into the epithelial cells, while the

SPI-2 is required for survival inside the cells. Once inside the epithelial cell, *Salmonella*, through effector proteins of the SPI-1 T3SS, initiate transcriptional reprogramming of both the host cells and of the bacteria that prevent activation of the innate immune receptors (Chen et al. 1996; Hobbie et al. 1997; Bruno et al. 2009). The bacterial transcriptional reprogramming results from the escape of some of intravascular bacteria into to the cytoplasm where they undergo hyper-replication before host cell lysis (Knodler 2015).

8.5.3 Evasion of Phagocyte Defense Mechanisms

Following invasion and penetration of the epithelial cell lining of the intestine, *Salmonella* reach the sub-epithelium where they encounter macrophages (Chappell et al. 2009; He et al. 2012; Braukmann et al. 2015). These macrophages can serve as both host cells and transport cells for the bacteria, so their ability to survive and replicate in the immune cells is of tantamount importance for persistence in extra-intestinal organs (Chappell et al. 2009; Wigley 2014). Survival, growth, and persistence in macrophages are dependent on the SPI-2 type III secretion system (Chappell et al. 2009; Setta et al. 2012a, b; He et al. 2012, 2013). In mammalian models, a second virulence system, the PhoP/Q two-component regulatory system, has also been shown to be a factor in *Salmonella* survival in macrophages (Thompson et al. 2011; Lathrop et al. 2015). However, there are no reports of the PhoP/Q system involvement in *Salmonella*-avian macrophage interactions. The SPI-2 virulence system is induced in *Salmonella* by intracellular signals as the bacteria are growing in the acidified, modified phagosome (*Salmonella*-containing vacuole [SCV]) which prevent phagolysosomal fusion and thus avoiding exposure to lysosomal antimicrobial contents (Vazquez-Torres et al. 2000; Haraga et al. 2008; Steele-Mortimer 2008). Furthermore, as yet unidentified specific SPI-2 effector proteins have been shown to protect *Salmonella* from the macrophage reactive oxygen intermediate, NADPH oxidase, and reactive nitrogen species, nitric oxide (NO) (Vazquez-Torres et al. 2000; Das et al. 2009; Aussel et al. 2011; Henard and Vazquez-Torres 2011). Specifically, *Salmonella* downregulates NO production and iNOS induction in IFN- γ-activated macrophages in a SPI-2-dependent manner (Das et al. 2009). Moreover, a SPI-2 effector protein blocks correct co-localization of NADPH oxidase vesicles with SCV (Vazquez-Torres et al. 2000; van der Heijden et al. 2015).

An emerging host defense mechanism against *Salmonella* is the process of autophagy. Autophagy, under normal homeostatic condition, is an evolutionarily conserved cellular response to remove defective proteins and organelles, but has been shown to be involved in the capture and removal of intracellular bacteria (Levine 2005; Cemma and Brumell 2012; Deretic et al. 2013). The autophagic response involves development of autophagosomes that engulf cytosolic components or bacteria that then fuse with lysosomes for degradation (Levine 2005). *Salmonella* have been shown to interact with and are contained by autophagy sys-

tems in both phagocytic and non-phagocytic host cells (Birmingham et al. 2006). In this process, during the formation of the SCV, cytoplasmic aggregates form that are ubiquitinated by host ligases that enables the aggregates to be recognized by the autophagy pathway (Mesquita et al. 2012; Narayanan and Edlemann 2014). Recent evidence has shown that *Salmonella* inhibits antibacterial autophagy through SPI-2-dependent effector proteins that target two different posttranslational protein modification pathways. Posttranslational modifications, such as ubiquitination and phosphorylation, play vital roles in bacterial evasion of phagocytic cell killing (Narayanan and Edlemann 2014). With the first, the intracellular bacteria release the SPI-2 effector protein, SseL, which deubiquinates the cytoplasmic aggregates which inhibits the ubiquitin-driven autophagy process in the macrophage (Mesquita et al. 2012). With the second mechanism, unknown SPI-2 factor(s) specifically phosphorylate the non-receptor tyrosine kinase, focal adhesion kinase (FAK), which in turn phosphorylates Akt, an upstream regulator of the serine/threonine kinase, mammalian target of rapamycin (mTOR). Activated Akt then phosphorylates mTOR, which, as a principle regulator of autophagy, suppresses the autophagic process (Owen et al. 2014). mTOR plays a vital role in cell growth and metabolism by sensing environmental cues, including when nutrients are in abundance and when immune cells are in metabolically demanding situations such as stimulation with growth factors, nutrient availability, and immune regulatory signals (Laplante and Sabatini 2012; Cobbald 2013).

Once survivability inside the macrophage is assured, *Salmonella* have been shown to influence the motility of the infected macrophage, thereby exploiting the macrophage as a Trojan horse to spread from the intestine to internal organs. Amazingly, the SPI-2 effector protein SseI appears to play a dual role in affecting macrophage motility both early during intestinal infection and later during the colonization of the internal organs (Worley et al. 2006). Following the resolution of an inflammatory response in the intestine, normal CD18+ cells (macrophages and dendritic cells) can reenter the bloodstream by traversing the basement endothelium in a process called reverse transmigration (Thornbrough and Worley 2012). Infected CD18+ cells do not normally reverse transmigrates, thus balancing resolving inflammation while inhibiting the spread of microbes. However, *Salmonella* actively exploits the reverse transmigration process by secreting SseI (also known as SrfH) that binds to the host protein, TRIP6, to stimulate reverse transmigration enhancing dissemination away from the intestine to internal organs (Worley et al. 2006; Thornbrough and Worley 2012). TRIP6 is an adaptor protein that regulates cellular motility (Yi et al. 2002; Lai et al. 2005). This is an extraordinary mechanism wherein "an intracellular pathogen overcomes host defenses designed to immobilize infected host cells" (Worley et al. 2006). Remarkably, once at the internal organs, SseI then plays a paradoxical role in maintaining a chronic infection (McLaughlin et al. 2009). Here, SseI *inhibits* phagocyte mobility by interacting with a different host regulator of cell migration, IQGAP1 (McLaughlin et al. 2009). Further, the authors also reported an SseI-dependent decrease of macrophage migration was also associated with a reduction in CD4+T cell numbers in the spleens of infected animals. Previous reports have demonstrated reduced T cell activation due to a SPI-2-dependent suppression of antigen presentation (Cheminay et al. 2005;

Tobar et al. 2006; Bueno et al. 2007). Therefore, the authors hypothesize that the data are suggestive that SseI indirectly controls CD4+T cell numbers by inhibiting migration of CD18+ cells and limiting their ability to effectively prime naïve T cells. Taken together, these results suggest reduced capacity of the host to clear *Salmonella* from extra-intestinal sites of infection consequently leading to asymptomatic long-term infection.

8.5.4 Subversion of Antimicrobial Peptides

Salmonella are able to subvert the antimicrobial peptide (AMP) killing activities of the intestinal innate immune system by a number of physical and genetic means (McKelvey et al. 2014; Matamouros and Miller 2015). For example, the bacteria are able to cloak their presence by remodeling their envelope thus increasing the hydrophobicity resulting in decreased binding of AMPs (Lee et al. 2004; Herrera et al. 2010; Kato et al. 2012). Further, *Salmonella* possess an outer membrane protease that can target and degrade cationic AMPs (Guina et al. 2000). *S. enteritidis* has two antimicrobial resistance genes, *virK* and *ybjX*, on its genome that confer bacterial resistance to polymyxin B and avian β-defensins (McKelvey et al. 2014). Both genes are part of the PhoP/PhoQ regulon and are involved in modulation of the outer membrane of the bacteria that results in resistance to AMPs.

8.5.5 Modification of PAMPs/Evasion of PRRs/Subvert PRR Signaling

Lipopolysaccharide (LPS) comprises the major portion of the gram-negative bacterial cell wall (Raetz and Whitfield 2002). LPS consists of three components: the lipid A domain, a core oligosaccharide, and a variable number of repeat units of a polysaccharide O antigen. In response to host signals, *Salmonella* is capable of modifying the lipid A portion of LPS by activating the PhoP/PhoQ and PmrA-PmrB regulons (Ernst et al. 2001; Raetz and Whitfield 2002; Kawasaki et al. 2005). Activation of these two-component regulatory systems results in the production of bacterial enzymes that palmitoylate, hydroxylate, deacylate, and attach aminoarabinose to lipid A (Ernst et al. 2001). These *Salmonella*-induced modifications increase resistance to AMPs and alter host recognition of LPS by TLR4 resulting in altering host cell signaling to mediate the innate immune response (Kawasaki et al. 2004a, b; Lee et al. 2004). Further, it has been found that the length of the O antigen, i.e., number of repeating units, is under genetic control of the bacteria. *S. enteritidis* appears to be able to increase the length of the O antigen component of the LPS molecule to increase colonization of the reproductive tract and increase bacterial survival in the egg during its formation (Coward et al. 2013).

Salmonella flagellin is required by the bacterium for motility (Stecher et al. 2004), but is also a target of two components of mammalian innate immune system:

(1) TLR5 which detects extracellular flagellin and (2) Naip5-Naip6/NirC4/caspase-1 which detects cytosolic flagellin (Gewirtz et al. 2001; Hayashi et al. 2001; Kofoed and Vance 2011; Miao et al. 2006). However, as of this writing, no reports in the literature poultry possess cytosolic PRR for the detection of flagellin. Flagellin production is tightly regulated, and as such expression can be altered by host environmental cues. *Salmonella* actively inhibits flagellin expression during the establishment of systemic infections as a means of avoiding immune detection (Stecher et al. 2004; Cummings et al. 2005; Lai et al. 2013).

Another mechanism by which *Salmonella* can evade detection by PRRs is to directly antagonize signaling components. For example, once a TLR is activated by a PAMP, an interaction between the Toll/interleukin-1 receptor (TIR) domains that are present on both the TLR and on adaptor proteins (MyD88 or TIRAP), which then activate specific signal transduction pathways in the host to generate a protective innate immune response (Patterson and Werling 2013). The importance of this interaction has been is obvious with the report that *Salmonella* possess a gene (*tipA*) that mimics the Toll/interleukin-1 receptor (TIR) domain of TLR and their adapter proteins that compete with the endogenous TIR domains and thus prevents downstream TLR4 signaling (Newman et al. 2006). Specifically, *Salmonella* TipA protein modulates NF-κB activation and IL-1β production (Newman et al. 2006, II 74:594).

Members of the cytosolic Nod-like receptor protein family (NLR) direct assembly of multiprotein complexes termed inflammasomes in response to detection of microbial products in the cytosol or disruption of cellular membranes by microbial virulence factors (Shin and Brodsky 2015; Storek and Monack 2015). Inflammasome assembly induces activation of caspase 1-dependent cleavage and secretion of IL-1 family cytokines and a caspase-1-dependent pro-inflammatory cell death (pyroptosis). Inflammasome activation plays a major role in host defense against a variety of pathogens, but a number of viral and bacterial pathogens have been found to interfere with inflammasome activation (Shin and Brodsky 2015). For example, it has recently been shown that pathogen-derived metabolites can be recognized by NLR resulting in the activation of inflammasome-mediated immunity (reviewed in Shin and Brodsky 2015). Specifically, the *Salmonella*-derived TCA cycle metabolite citrate is recognized by the NLRP3 inflammasome resulting extra-intestinal clearance of a systemic infection (Wynosky-Dolfi et al. 2014). However, screening a *S. typhimurium* transposon library, it was found that three *Salmonella* genes that code for TCA cycle enzymes active during intracellular infection (*acnB*, aconitase; *aceA*, isocitrate lyase; *icdA*, isocitrate dehydrogenase) were found to modulate inflammasome activation through the metabolism of citrate (Wynosky-Dolfi et al. 2014).

8.6 Targeting Host Signaling Cascades

Salmonella use effector proteins to divert, inhibit, and otherwise influence host cell signaling pathways to the advantage of the bacteria obstructing immune signaling pathways like the transcription factor NF-κB and the mitogen-activated protein kinase (MAPK) signaling cascade (Haraga and Miller 2002; Collier-Hyams et al.

2002; Le Negrate et al. 2008; Mazurkiewicz et al. 2008; Wu et al. 2012). Both NF-κB and MAPK activations lead to transcription of pro-inflammatory cytokines and antimicrobial molecules genes.

8.6.1 Target NF-κB

AvrA is an effector protein that functions as an immunological brake inhibiting the activation of NF-κB by stabilizing two inhibitors of NF-κB pathway, IκBα and β-catenin, which prevents release of the NF-κB for translocation to the nucleus, thereby inhibiting the inflammatory responses (Collier-Hyams et al. 2002; Ye et al. 2007, Am. J. Pathol. 171:882). Similarly, the effector protein *Salmonella* secreted factor L (SseL), a deubiquitinase that suppresses NF-κB activation by removing ubiquitin from IκBα preventing NF-κB translocation (Le Negrate et al. 2008). Another effector protein, SspH1, localizes to the host cell nucleus and inhibits NF-κB-dependent gene expression (Haraga and Miller 2002).

8.6.2 Target MAPK

AvrA has also shown to possess acetyltransferase activity that targets upstream kinases of the c-Jun-NH2-terminal kinase (JNK) pathway, thereby suppressing apoptotic removal of the bacterial intracellular niche and avoiding acquired immune mechanisms (Wu et al. 2012). SpvC inhibits inflammation by the dephosphorylation of the extracellular signal-regulated kinase (ERK) signaling pathway (Mazurkiewicz et al. 2008).

8.6.3 Macrophage Polarization: A Salmonella *Immunometabolic Survival Niche*

Upon recruitment into tissues, mononuclear phagocytes respond to local environmental signals (pro- or anti-inflammatory cytokines, microbial products, dead and/or damaged cells, tissue metabolism, activated lymphocytes) by changing their physiology to acquire distinct functional phenotypes (Gordon and Martinez 2010; Biswas and Mantovani 2010), specifically the so-called “classically activated” M1 macrophages and the “alternatively activated” M2 macrophages (Biswas and Mantovani 2010; Sica and Mantovani 2012). The terminology is based on the Th1- and Th2-derived immune responses (Mills et al. 2000). Although the Th1/Th2 paradigm has been defined in chickens (Guo et al. 2013; Chausse et al. 2014), there is no direct evidence that chicken macrophages can polarize into the M1/M2

phenotypes (Wigley 2014). However, *Salmonella* does appear to prefer the M2 phenotype macrophage for long-term persistent infections of both murine and human macrophages (Eisele et al. 2013; Lathrop et al. 2015).

We and others have recently demonstrated the development of a Th2, anti-inflammatory response in the cecum of chickens that begins at least 4 days after an initial infection with *Salmonella* and continues for weeks (Chausse et al. 2014; Kogut et al. 2015a, b). Further, we have noted a significant increase in T regulatory cells in the cecum that corresponds to this shift from a pro-inflammatory to an anti-inflammatory environment (Shanmugasundaram et al. 2015). Lastly, we also found alterations in the metabolic signatures of the cecum of the *Salmonella*-infected animals that are linked to a M2 phenotype, albeit, in a tissue and not macrophages. However, macrophage polarization is linked with dramatic alterations in multiple metabolic pathways (Biswas and Mantovani 2013; Shapiro et al. 2011). Specifically, lipid oxidation metabolism mediated by the peroxisome proliferator-activated receptors (PPAR)γ/δ pathways within the M2 macrophage provides advantageous niche for a number of intracellular microbial pathogens including *S. typhimurium* in mice (Eisele et al. 2013), *Mycobacterium tuberculosis* (Almeida et al. 2012), *Brucella abortus* (Xavier et al. 2013), *Listeria monocytogenes* (Abdullah et al. 2012), *Francisella tularensis* (Shirey et al. 2008), *Leishmania* (Chan and Fong 2012), and *Toxoplasma gondii* (Jensen et al. 2010). We submit that a persistent, carrier-state *Salmonella* infection in the chicken cecum induces a number of environmental cues that can potentially alter the polarization of infiltrating mononuclear phagocytes from a M1 state early infection to a preferential M2 state. The M2 macrophages "represent a unique niche for long-term intracellular bacterial survival" (Eisele et al. 2013) as well as an excellent mechanism for: (a) evading the host immune response, (b) promoting bacterial replication, and (c) dissemination throughout the reticuloendothelial system to internal organs.

References

Abdullah, Z., Geiger, S., Nini-Castro, A., Bottcher, J. P., Muroliv, E., Gardt, M., et al. (2012). Lack of PPARγ in myeloid cells confers resistance to *Listeria monocytogenes* infection. *PLoS One, 7*, e37349.

Aehle, S., & Curtiss III, R. (2017). Current and future perspectives on development of *Salmonella* vaccine technologies. In S. C. Ricke & R. K. Gast (Eds.), *Producing safe eggs: Microbial ecology of Salmonella* (pp. 281–300). New York: Academic Press.

Almeida, P. B., Carneiro, A. B., Silva, A. C., & Bozza, P. T. (2012). PPARγ expression and function in mycobacterial infections: Role in lipid metabolism, immunity, and bacterial killing. *PPAR Research, 2012*, 383829.

Ashida, H., Ogawa, M., Kim, M., Mimuro, H., & Sasakawa, C. (2012). Bacteria and host interactions in the gut epithelial barrier. *Nature Chemical Biology, 8*, 36–45.

Aussel, L., Zhao, W., Hebard, M., Galhon, A.-A., Vala, J. P. M., Henri, S., et al. (2011). *Salmonella* detoxifying enzymes are sufficient to cope with host oxidative burst. *Molecular Microbiology, 80*, 628–640.

Avraham, R., Haseley, N., Brown, D., Penaranda, C., Jilon, H. B., Tromnetta, J. J., et al. (2015). Pathogen cell-to-cell variability drives heterogeneity in host immune responses. *Cell, 162*, 1309–1321.

Babu, U. S., Gaines, D. W., Lillehoj, H., & Raybourne, R. B. (2006). Differential reactive oxygen and nitrogen production and clearance of *Salmonella* serovars by chicken and mouse macrophages. *Developmental and Comparative Immunology, 30*, 942–953.

Barrow, P. A. (2007). *Salmonella* infections: Immune and non-immune protection with vaccines. *Avian Pathology, 36*, 1–13.

Barrow, P. A., Morrow, M. A., Prost, A. J., & Bland, P. (1997). Prevention of salmonellosis in gnotobiotic pigs by pre-colonization with avirulent Salmonella strain. In: *Proceedings of the International Symposium on Salmonella and Salmonellosis* (pp. 519–520). Zoopole Development, Institut Superier des Productions Animales et Industries Agro-alimentaires.

Baurhoo, B., Ferket, P. R., & Zhao, X. (2009). Effect of diets containing different concentrations of mannan oligosaccharide or antibiotics on growth performance, intestinal development, cecal and litter microbial populations, and carcass parameters of broilers. *Poultry Science, 88*, 2162–2172.

Beal, R. K., Powers, C., Davison, T. F., Barrow, P. A., & Smith, A. I. (2006). Clearance of *Salmonella enterica serovar* Typhimurium is independent of B-cell function. *Infection and Immunity, 74*, 1442–1444.

Beal, R. K., Powers, C., Wigley, P., Barrow, P. A., & Smith, A. I. (2004a). Temporal dynamics of the cellular, humoral, and cytokine responses in chickens during primary and secondary infection with *Salmonella enterica* serovar Typhimurium. *Avian Pathology, 33*, 25–33.

Beal, R. K., Wigley, P., Powers, C., Hulme, S. D., Barrow, P. A., & Smith, A. I. (2004b). Age at primary infection with *Salmonella enterica* serovar Typhimurium in the chicken influences persistence of infection and subsequent immunity to re-challenge. *Veterinary Immunology and Immunopathology, 100*, 151–164.

Berndt, A., & Methner, U. (2001). Gamma/delta T cell response of chickens after oral administration of attenuated and non-attenuated Salmonella typhimurium strains. *Veterinary Immunology and Immunopathology, 78*, 143–161.

Berndt, A., Pieper, L., & Methner, U. (2006). Circulating gamma delta T cells in response to *Salmonella enterica* serovar Enteritidis exposure in chickens. *Infection and Immunity, 74*, 3967–3978.

Berndt, A., Wilheim, A., Jugert, C., Pieper, J., Sachse, K., & Methner, U. (2007). Chcike cecum immune response to *Salmonella enterica* serovars of different levels of invasiveness. *Infection and Immunity, 75*, 5993–6007.

Birmingham, C. L., Smith, A. C., Bakowski, M. A., Yoshimori, T., & Brumell, J. H. (2006). Autophagy controls *Salmonella* infection in response to damage to the *Salmonella*-containing vacuole. *The Journal of Biological Chemistry, 281*, 11374–11383.

Biswas, S. K., & Mantovani, A. (2010). Macrophage plasticity and interaction with lymphocyte subsets: Cancer as a paradigm. *Nature Immunology, 11*, 889–896.

Biswas, S. K., & Mantovani, A. (2013). Orchestration of metabolism by macrophage. *Cell Metabolism, 15*, 432–437.

Bohez, L., Dewulf, J., Ducatelle, R., Pasmans, F., Haesbrouck, F., & van Immerseel, F. (2008). The effect of oral administration of homologous hilA mutant strain on long-term colonization and transmission of *Salmonella Enteritidis* in broiler chickens. *Vaccine, 26*, 372–378.

Braukmann, M., Barrow, P. A., Berndt, A., & Methner, U. (2016). Combination of competitive exclusion and immunization with a live *Salmonella* vaccine in newly hatched chickens: Immunological and microbiological effects. *Research in Veterinary Science, 107*, 34.

Braukmann, M., Methner, U., & Berndt, A. (2015). Immune reaction and survivability of *Salmonella* Typhimurium and *Salmonella* Infantis after infection of primary avian macrophages. *PLoS One, 10*, e0122540.

Brown, E. M., Wlodarska, M., Willing, B. P., Vonaesch, P., Han, J., Reynolds, L. A., et al. (2015). Diet and specific microbial exposure trigger, features of environmental enteropathy in a novel murine model. *Nature Communications, 6*, 7806.

Bruno, V. M., Hannemann, S., Tejero, M. L., Flavell, R. A., & Galan, J. E. (2009). *Salmonella* Typhimurium type III secretion effectors stimulate innate immune responses in cultured epithelial cells. *PLoS Pathogens, 5*, e1000538.

Bueno, S. M., Gonzales, P. A., Schwebach, J. R., & Kalergis, A. M. (2007). T cell immunity evasion by *Salmonella enterica*. *Immunology Letters, 111*, 14–20.

Burton, N. A., Schumann, N., Casse, O., Steeb, A. K., Claudi, B., Zanid, J., et al. (2014). Disparate impact of oxidative host defenses determines the fate of *Salmonella* during systemic infection in mice. *Cell Host & Microbe, 15*, 74–83.

Cemma, M., & Brumell, J. H. (2012). Interactions of pathogenic bacteria with autophagy systems. *Current Biology, 22*, R540–R545.

Chan, M. M., & Fong, D. (2012). The interplay of PPARs with parasites and related intracellular pathogens. *PPAR Research, 2012*, 624845.

Chappell, L., Kaiser, P., Barrow, P. A., Jones, M. A., Johnson, C., & Wigley, P. (2009). The immunobiology of avian systemic salmonellosis. *Veterinary Immunology and Immunopathology, 128*, 53–59.

Chausse, A.-M., Grepinet, O., Bottreau, E., Robert, V., Hennequet-Antier, C., Lalmanach, A.-C., et al. (2014). Susceptibility to *Salmonella* carrier-state: A possible Th2 response in susceptible chickens. *Veterinary Immunology and Immunopathology, 159*, 16–28.

Chee, S. H., Iji, P. A., Choct, M., Mikkelsen, L. L., & Kocher, A. (2010). Characterization and response of intestinal microflora and mucins to mannooligosaccharide and antibiotic supplementation in broiler chickens. *British Poultry Science, 51*, 368–380.

Cheminay, C., Mohlenbrink, A., & Hensel, M. (2005). Intracellular *Salmonella* inhibit antigen presentation by dendritic cells. *Journal of Immunology, 174*, 2892–2899.

Chen, L.-M., Hobbie, S., & Galan, J. E. (1996). Requirement of CD42 for *Salmonella*-induced cytoskeletal and nuclear responses. *Science, 274*, 2115–2118.

Cobbald, S. P. (2013). The mTOR pathway and integrating immune regulation. *Immunology, 140*, 391–398.

Coe, N. E., Frank, D. E., Wood, R. L., & Roth, J. A. (1992). Alteration of neutrophil function in BCG-treated and non-treated swine after exposure to *Salmonella typhimurium*. *Veterinary Immunology and Immunopathology, 33*, 37–50.

Collier-Hyams, L. S., Zeng, H., Sun, J., Tomlinson, A. D., Bao, Z. Q., Chen, H. C., et al. (2002). *Salmonella* AvrA effector inhibits the key pro-inflammatory, anti-apoptotic NF-κB pathway. *Journal of Immunology, 169*, 2846–2850.

Conlan, J. W., & North, R. J. (1992). Early pathogenesis of infection in the liver with the facultative intracellular bacteria *Listeria monocytogenes, Francisella tularensis,* and *Salmonella typhimurium* involves lysis of infected hepatocytes by leucocytes. *Infection and Immunity, 60*, 5164–5171.

Coward, C., Sait, L., Cogan, T., Humphrey, T. J., & Maskell, D. J. (2013). O-antigen repeat number in *Salmonella enterica* serovar Enteritidis is important for egg contamination, colonization of the chicken reproductive tract and survival in egg albumin. *FEMS Microbiology Letters, 343*, 169–176.

Cummings, L. A., Barrett, S. L. R., Wilerson, W. D., Fellnerova, I., & Cookson, B. T. (2005). FLiC-specific CD4+ T cell responses are restricted by bacterial regulation of antigen expression. *The Journal of Immunology, 174*, 7929–7938.

Das, P., Lahiri, A., Lahiri, A., & Chakravortty, D. (2009). Novel role of the nitrite transporter NirC in *Salmonella* pathogenesis: SPI2-dependent suppression of inducible nitric oxide synthase in activated macrophages. *Microbiology, 155*, 2476–24889.

De Cort, W., Geeraerts, S., Balan, V., Elroy, M., Haesbrouck, F., Ducatelle, R., et al. (2013). A *Salmonella* Enteritidis *hilAssrAfliG* deletion mutant is a safe live vaccine strain that confers protection against colonization by *Salmonella* Enteritidis in broilers. *Vaccine, 31*, 5104–5110.

De Cort, W., Haesbrouck, F., Ducatelle, R., & Van Immerseel, F. (2015). Administration of a *Salmonella* Enteritidis *hilAssrAfliG* strain by coarse spray to newly hatched broilers reduces colonization and shedding of a *Salmonella* Enteritidis challenge strain. *Poultry Science, 94*, 131–135.

Deretic, V., Saitoh, T., & Akira, S. (2013). Autophagy in infection, inflammation, and immunity. *Nature Reviews. Immunology, 13*, 722–737.

Desin, T. S., Koster, W., & Potter, A. A. (2013). *Salmonella* vaccines in poultry: Past, present, and future. *Expert Review of Vaccines, 12*, 87–96.

Desmidt, M., Ducatelle, R., & Haesebrouck, F. (1997). Pathogenesis of *Salmonella enteritidis* phage-type four after experimental infection of young chickens. *Veterinary Microbiology, 56*, 99–109.

Dieye, Y., Ameiss, K., Mellata, M., & Curtiss III., R. (2009). The *Salmonella* pathogenicity island (SPI)-1 contributes more than SPI2 to the colonization of the chicken by *Salmonella enterica* serovars Typhimurium. *BMC Microbiology, 9*, 3.

Dinev, I. (2009). Clinical and morphological investigations on the prevalence of lameness associated with femoral head necrosis in broilers. *British Poultry Science, 50*, 284–290.

Eisele, N. A., Ruby, T., Jacobson, A., Manzanillo, P. S., Cox, J. S., Lam, L., et al. (2013). *Salmonella* require the fatty acid regulator PPARɣ for the establishment of a metabolic environment essential for long-term persistence. *Cell Host & Microbe, 14*, 171–182.

Eriksson, S., Lucchini, B., Thompson, A., Rhen, M., & Hilton, J. C. (2003). Unveiling the biology of macrophage infection by gene expression profiling of intracellular *Salmonella enterica*. *Molecular Microbiology, 47*, 103–118.

Ernst, R. K., Guina, T., & Miller, S. L. (2001). *Salmonella typhimurium* outer membrane remodeling role in resistance to host innate immunity. *Microbes and Infection, 3*, 1327–1334.

Foster, N., Lovell, M. A., Marston, K. L., Hulme, S. D., Frost, A. J., Bland, P., et al. (2003). Rapid protection of gnotobiotic pigs against experimental salmonellosis following induction of polymorphonuclear leukocytes by avirulent *Salmonella enterica*. *Infection and Immunity, 71*, 182–191.

Galán, J. E., & Collmer, A. (1999). Type III secretion machines: Bacterial devises for protein delivery into host cells. *Science, 284*, 1322–1328.

Galan, J. E., & Wolf-Watz, H. (2006). Protein delivery into eukaryotic cells by type III secretion machines. *Nature, 444*, 567–573.

Gantois, I., Ducatelle, R., Pasmans, F., Haiesbrouck, F., Gast, R., Humphrey, T. J., et al. (2006). Mechanisms of egg contamination by *Salmonella* Enteritidis. *FEMS Microbiology Reviews, 33*, 718–738.

Gast, R. K., & Benson, S. T. (1995). The comparative virulence for chicks of *Salmonella enteritidis* phage type 4 isolates and isolated of phage types commonly found in poultry in the United States. *Avian Diseases, 39*, 567–574.

Genovese, K. J., He, H., Swaggerty, C. L., & Kogut, M. H. (2013). The avian heterophil. *Developmental and Comparative Immunology, 41*, 334–340.

Gewirtz, A. T., Navas, T. A., Lyons, S., Godowski, P. J., & Madera, J. A. (2001). Bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial pro-inflammatory gene expression. *Journal of Immunology, 167*, 1882–1885.

Gordon, S. (2005). Alternative activation of macrophages. *Nature Reviews. Immunology, 3*, 23–35.

Gordon, S., & Martinez, F. O. (2010). Alternative activation of macrophages: Mechanism and functions. *Immunity, 32*, 593–604.

Guina, T., Yi, E. C., Wang, H., Hackett, M., & Miller, S. I. (2000). A PhoP-regulated outer membrane protease of *Salmonella enterica* serovars Typhimurium promotes resistance to alpha-helical antimicrobial peptides. *Journal of Bacteriology, 182*, 4077–4086.

Guo, P., Thomas, J. D., Bruce, M. P., Hilton, T. M., Bean, A. G. D., & Lowentahl, J. W. (2013). The chicken Th1 response: Potential therapeutic applications of ChIFN-ɣ. *Developmental and Comparative Immunology, 41*, 389–396.

Hand, T. W. (2016). The role of the microbiota in shaping infectious immunity. *Trends in Immunology, 37*, 647–658.

Hand, T. W., Dos Santos, L. M., Bouladoux, N., Malloy, M. J., Agan, A. J., Pepper, M., et al. (2012). Acute gastrointestinal infection induces long-lived microbiota specific cell responses. *Science, 337*, 1553–1556.

Haraga, A. I., & Miller, S. I. (2002). A *Salmonella enterica* serovars Typhimurium translocated leucine-rich repeat effector protein inhibits NF-κB-dependent gene expression. *Infection and Immunity, 71*, 4052–4058.

Haraga, A. I., Olson, M. B., & Miller, S. I. (2008). Salmonellae interplay with host cells. *Nature Reviews. Microbiology, 6*, 53–66.

Hayashi, F., Smith, K. D., Ozinsky, A., Dawn, T. R., Yi, E. C., Goodlet, D. R., et al. (2001). The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature, 410*, 1099–1103.

He, H., Genovese, K. J., & Kogut, M. H. (2012). A comparative study on invasion, survival, and modulation of oxidative burst and nitric oxide responses of macrophages (HD11), and systemic infection in chickens by prevalent poultry *Salmonella* serovars. *Foodborne Pathogens and Disease, 9*, 1104–1110.

He, H., Genovese, K. J., Swaggerty, C. L., Nisbet, D. J., & Kogut, M. H. (2007). In vivo priming heterophil innate immune functions and increasing resistance to *Salmonella enteritidis* infection in neonatal chickens by immune stimulatory CpG oligodeoxynucleotides. *Veterinary Immunology and Immunopathology, 117*, 275–283.

He, H., Genovese, K. J., Swaggerty, C. L., Nisbet, D. J., & Kogut, M. H. (2013). Nitric oxide is a biomarker of intracellular *Salmonella* viability and identification of the bacteriostatic activity of protein kinase A inhibitor H-89. *PLoS One, 8*, e58873.

He, H., McacKinnon, K. M., Genovese, K. J., & Kogut, M. H. (2011). CpG oligodeoxynucleotide and double stranded RNA synergize to enhance nitric oxide production and mRNA expression of inducible nitric oxide synthase, pro-inflammatory cytokines and chemokines in chicken monocytes. *Innate Immunity, 17*, 137–144.

Helaine, S., Thompson, J. A., Watson, K. G., Liu, M., Boyle, C., & Holden, D. W. (2010). Dynamics of intracellular bacterial replication at the single cells level. *PNAS, 107*, 3746–3751.

Henard, C. A., & Vazquez-Torres, A. (2011). Nitric oxide and *Salmonella* pathogenesis. *Frontiers in Microbiology, 2*, 84.

Hensel, M. (2000). *Salmonella* pathogenicity island 2. *Molecular Microbiology, 36*, 1015–1023.

Herrera, C. M., Hankins, J. V., & Trent, M. S. (2010). Activation of PmrA inhibits LpxT-dependent phosphorylation of lipid A promoting resistance to antimicrobial peptides. *Molecular Microbiology, 76*, 1444–1460.

Heyndrickx, M., Vandekerchove, D., Herman, L., Rollier, I., Grijspeerdt, K., & De Zutter, L. (2002). Routes for *Salmonella* contamination of poultry meat: Epidemiological study from hatchery to slaughterhouse. *Epidemiology and Infection, 129*, 253–265.

Hobbie, S., Chen, L. M., Davis, R. J., & Galan, J. E. (1997). Involvement of mitogen-activated protein kinase pathways in the nuclear responses and cytokine production induced by *Salmonella typhimurium* in cultured epithelial cells. *Journal of Immunology, 159*, 5550–5559.

Hooper, L. V., Littman, D. R., & MacPherson, A. J. (2012). Interactions between the microbiota and the immune system. *Science, 336*, 1268–1273.

Imre, A., Szmolka, A., Olasz, F., & Nagy, B. (2015). Vaccine potential of a nonflagellated, virulence-plasmid cured (fliD-, pSEVΔ) mutant of *Salmonella* Enteritidis for chickens. *Acta Veterinaria Hungarica, 63*, 285–302.

Jawale, C. V., & Lee, J. H. (2014). Characterization of adaptive immune responses induced by a new genetically inactivated *Salmonella* Enteritidis vaccine. *Comparative Immunology, Microbiology and Infectious Diseases, 37*, 159–167.

Jawale, C. V., & Lee, J. H. (2016). Evaluation of immunogenicity and protective efficacy of adjuvanted *Salmonella* Typhimurium ghost vaccine against salmonellosis in chickens. *The Veterinary Quarterly, 36*, 130–136.

Jensen, K., Gallagher, I. J., Kaliszewska, A., Zhang, C., Abejide, O., Gallagher, M. P., et al. (2016). Live and inactivated *Salmonella enterica* serovar Typhimurium stimulate similar but, distinct transcriptome profiles in bovine macrophages and dendritic cells. *Veterinary Research, 47*, 46.

Jensen, K. D. C., Wang, Y., Tait Wojno, E. D., Shashi, A. J., Hu, K., Comel, L., et al. (2010). Toxoplasma polymorphic effectors determine macrophage polarization and intestinal inflammation. *Cell Host & Microbe, 9*, 472–483.

Jeon, B. W., Nandre, R. M., & Lee, J. H. (2013). Immunization with an attenuated *Salmonella* Gallinarum mutant as a fowl typhoid vaccine with a live adjuvant strain secreting the B subunit of *Escherichia coli* heat labile enterotoxin. *BMC Veterinary Research, 9*, 96.

Jesudhasan, P. R., McReynolds, J. L., He, H., Genovese, K. J., Drolesky, R., Swaggerty, C. L., et al. (2015). Electron-beam inactivated vaccine against *Salmonella enteritidis* colonization in molting hens. *Avian Diseases, 59*, 165–170.

Johanns, T. M., Ertell, J. M., Rowe, J. H., & Way, S. S. (2010). Regulatory T cell responses suppressive potency dictates the balance between bacterial proliferation and clearance during persistent *Salmonella* infection. *PLoS Pathogens, 6*, e1001043.

Kamble, N. M., & Lee, J. H. (2016). Characterization and evaluation of a *Salmonella enterica* serotype Senftenberg mutant created by deletion of virulence-related genes for use as a live attenuated vaccine. *Clinical and Vaccine Immunology, 23*, 802–812.

Kato, A., Chen, H. D., Lafiti, T., & Grossman, E. A. (2012). Reciprocal control between a bacterium's regulatory system and the modification status of its polysaccharide. *Molecular Cell, 47*, 897–908.

Kawasaki, K., Ernst, R. K., & Miller, S. I. (2004a). 3-O-deacylation of lipid A by PagL, a PhoP/PhoQ-regulated deacylase of *Salmonella typhimurium*, modulates signaling through Toll-like receptor 4. *The Journal of Biological Chemistry, 279*, 20044–22248.

Kawasaki, K., Ernst, R. K., & Miller, S. I. (2004b). Deacylation and palmitoylation of lipd A by salmonellae outer membrane enzymes modulate host signaling through Toll-like receptor 4. *Journal of Endotoxin Research, 10*, 439–444.

Kawasaki, K., Ernst, R. K., & Miller, S. I. (2005). Inhibition of *Salmonella enterica* serovars Typhimurium lipopolysaccharide deacylation by aminoarabinose membrane modification. *Journal of Bacteriology, 187*, 2448–2457.

Knodler, L. A. (2015). *Salmonella enterica*: Living a double life in epithelial cells. *Current Opinion in Microbiology, 23*, 23–31.

Kofoed, E. M., & Vance, R. E. (2011). Innate immune recognition of bacterial ligands by NAPs determines inflammasome specificity. *Nature, 477*, 592–595.

Kogut, M. H., & Arsenault, R. J. (2015). A role for the non-canonical Wnt-β-catenin and TGF-β signaling pathways in the induction of tolerance during the establishment of *Salmonella enterica* serovar Enteritidis persistent cecal infection in chickens. *Frontiers in Veterinary Science, 2*, 33.

Kogut, M. H., & Arsenault, R. J. (2017). Immunometabolic phenotype alterations associated with the induction of disease tolerance and persistent asymptomatic infection of *Salmonella* in the chicken intestine. *Frontiers in Immunology, 8*, 372.

Kogut, M. H., Genovese, K. J., He, H., & Arsenault, R. J. (2015b). AMPK and mTOR: Sensors and regulators of immunometabolic changes during *Salmonella* infection in the chicken. *Poultry Science, 95*, 345–353.

Kogut, M. H., McGruder, E. D., Hargis, B. M., Williams, J. D., Corrier, D. E., & DeLoach, J. R. (1994a). Dynamics of avian inflammatory response to *Salmonella*-immune lymphokines. Changes in avian blood leukocyte populations. *Inflammation, 18*, 373–388.

Kogut, M. H., McGruder, E. D., Hargis, B. M., Williams, J. D., Corrier, D. E., & DeLoach, J. R. (1995). In vivo activation of heterophils function in chickens following injection with *Salmonella enteritidis*-immune lymphokines. *Journal of Leukocyte Biology, 57*, 56–62.

Kogut, M. H., Swaggerty, C. L., Chiang, H. I., Genovese, K. J., He, H., Zhou, H., et al. (2015a). Critical role of glycogen synthase kinase-3β in regulating the avian heterophil response to *Salmonella enterica* serovars Enteritidis. *Frontiers in Veterinary Science, 1*, 10.

Kogut, M. H., Tellez, G. I., Hargis, B. M., Williams, J. D., Corrier, D. E., & DeLoach, J. R. (1993). The effect of 5-fluorouracil treatment of chicks: A cell depletion model for the study of avian polymorphonuclear leukocytes and natural host defenses. *Poultry Science, 72*, 1873–1880.

Kogut, M. H., Tellez, G. I., McGruder, E. D., Hargis, B. M., Williams, J. D., Corrier, D. E., et al. (1994b). Heterophils are decisive components in the early responses of chickens to *Salmonella enteritidis* infections. *Microbial Pathogenesis, 16*, 141–151.

Lai, Y.-U., Chen, C.-S., Lin, W.-C., & Lin, F. T. (2005). C-Src-mediated phosphorylation of TRIP6 regulates its function in lysophosphatidic acid-induced cell migration. *Molecular and Cellular Biology, 25*, 5859–5868.

Lai, M. A., Quarles, E. K., Lopez-Yglesius, A. H., Zhao, X., Haijar, A. M., & Smith, K. D. (2013). Innate immune detection of flagellin positively and negatively regulates *Salmonella* infection. *PLoS One, 8*, e72047.

Lalsiamthara, J., & Lee, J. H. (2016). Virulence associated genes-deleted *Salmonella* Montevideo is attenuated, highly immunogenic and confers protection against virulent challenge in chickens. *Frontiers in Microbiology, 7*, 1634.

Lan, Y., Verstegen, M. W. A., Tammings, S., & Williams, B. A. (2005). The role of the commensal gut microbial community in broiler chickens. *World's Poultry Science Journal, 61*, 105–112.

Laplante, M., & Sabatini, D. M. (2012). mTOR signaling in growth control and disease. *Cell, 149*, 274–293.

Lathrop, S. K., Binder, K. A., Starr, T., Cooper, K. G., Chong, A., Carmody, A. B., et al. (2015). Replication of *Salmonella enterica* serovar Typhimurium in human monocyte-derived macrophages. *Infection and Immunity, 83*, 2661–2667.

Le Negrate, G., Faustin, B., Welsh, K., Loeffler, M., Krajewska, M., Hasegawa, P., et al. (2008). *Salmonella* secreted factor L deubiquitinase of *Salmonella typhimurium* inhibits NF-κB, suppresses IκB ubiquitination and modulates innate immune responses. *Journal of Immunology, 180*, 5045–5056.

Lee, H., Hsu, F. F., Turk, J., & Groisman, E. A. (2004). The PmrA-regulated pmrC gene mediates phosphoethanolamine modification of lipid A and polymyxin resistance in *Salmonella enterica*. *Journal of Bacteriology, 186*, 4124–4133.

Levine, B. (2005). Eating oneself and uninvited guests: Autophagy-related pathways in cellular defense. *Cell, 120*, 159–162.

Lillehoj, E. P., Yun, C. H., & Lillehoj, H. S. (2000). Vaccines against the avian enteropathogens *Eimeria, Cryptosporidium,* and *Salmonella*. *Animal Health Research Reviews, 1*, 47–65.

Lin, F. R., Wang, X. M., Hsu, H. S., Mumaw, V. R., & Nakoneczna, I. (1987). Electron microscopic studies on the location of bacterial proliferation in the liver in murine salmonellosis. *British Journal of Experimental Pathology, 68*, 539–550.

Matamouros, S., & Miller, S. I. (2015). *S. Typhimurium* strategies to resist killing by cationic antimicrobial peptides. *Biochimica et Biophysica Acta, 1848*, 3021–3025.

Matulova, M., Havlickova, H., Sisak, F., & Rychlik, I. (2013). Vaccination of chickens with SPI1-Ion and SPI-Ion-fliC mutant of *Salmonella enterica* serovars Enteritidis. *PLoS One, 8*, e66172.

Mazurkiewicz, S., Thomas, J., Thompson, J. A., Liu, M., Arbibe, L., Sansonetti, P., et al. (2008). SpvC is a *Salmonella* effector with phosphothreonine lyase activity on host mitogen-activated protein kinases. *Molecular Microbiology, 67*, 1371–1383.

McIntrye, J., Rowley, D., & Jenkin, C. R. (1967). The functional heterogeneity of macrophages at the single cell level. *Australian Journal of Experimental Biology and Medical Science, 45*, 675–680.

McKelvey, J. A., Yang, M., Jiang, Y., & Zhang, S. (2014). *Salmonella enterica* serovar Enteritidis antimicrobial peptide resistance genes aid in defense against chicken innate immunity, fecal shedding, and egg deposition. *Infection and Immunity, 82*, 5185–5202.

McLaughlin, L. M., Govoni, G. R., Gerke, C., Gopinath, S., Perry, K., Laidlaw, G., et al. (2009). The *Salmonella* SPI2 effector SseI mediates long-term systemic infection by modulating host cell migration. *PLoS Pathogens, 5*, e1000671.

Mesquita, S., Thomas, M., Sachee, M., Santos, A. J., Figueira, R., & Holden, D. W. (2012). The *Salmonella* deubiquitinase SseL inhibits selective autophagy of cytosolic aggregates. *PLoS Pathogens, 8*, e1002743.

Methner, U., haase, A., Berndt, A., Martin, G., Nagy, B., & Barrow, P. A. (2011). Exploitation of intestinal colonization-inhibition between salmonella organisms for live vaccines in poultry: Potential and limitations. *Zoonoses and Public Health, 58*, 343–357.

Miao, E. A., Alpuche-Aranda, C. M., Dors, M., Clark, A. E., Bader, M. W., Miller, S. I., et al. (2006). Cytoplasmic flagellin activates caspase-1 and secretion of interleukin-1β via Ipaf. *Nature Immunology, 7*, 569–575.

Mills, C. D., Kincaid, K., Alt, J. M., Heilman, M. J., & Hill, A. M. (2000). Cellular immunology and immune regulation of M-1/M-2 macrophages and the Th1/Th2 paradigm. *Journal of Immunology, 164*, 6166–6173.

Mosser, D. M., & Edwards, J. P. (2008). Exploring the full spectrum of macrophage activation. *Nature Reviews. Immunology, 8*, 958–969.

Muniz, E. C., Verdi, R., Leao, J. A., Back, A., & Nascimento, V. P. (2017). Evaluation of the effectiveness and safety of a genetically modified live vaccine in broilers challenged with Salmonella Heidelberg. *Avian Pathology, 46*, 676. https://doi.org/10.1080/03079457.2017.1348598

Nandre, R. M., & Lee, J. H. (2014). Construction of a recombinant attenuated *Salmonella* Enteritidis strain secreting *Escherichia coli* heat-labile enterotoxin B subunit protein and its immunogenicity and protection efficacy against salmonellosis in chickens. *Vaccine, 32*, 425–431.

Narayanan, L. A., & Edlemann, M. J. (2014). Ubiquitination as an efficient molecular strategy employed in *Salmonella* infection. *Frontiers in Immunology, 5*, 558.

Newman, R. M., Salunkhe, P., Godzik, A., & Reed, J. C. (2006). Identification and characterization of a novel bacterial virulence factor that shares homology with mammalian Toll/interleukin-1 receptor family proteins. *Infection and Immunity, 74*, 594–601.

O'Brien, S. J. (2013). The 'decline and fall' of nontyphoid salmonella in the United Kingdom. *Clinical Infectious Diseases, 56*, 705–710.

Oakley, B. B., & Kogut, M. H. (2016). Spatial and temporal changes in the broiler chicken cecal and fecal microbiomes and correlations of bacterial taxa with cytokine gene expression. *Frontiers in Veterinary Science, 3*, 11.

Owen, K. A., Meyer, C. B., Bouton, A. H., & Casanova, J. E. (2014). Activation of focal adhesion kinases by *Salmonella* suppresses autophagy via an Akt/mTOR signaling pathway and promotes survival in macrophages. *PLoS Pathogens, 10*, e1004159.

Park, S. H., Kim, S. A., Rubinelli, P. M., Roto, S. M., & Ricke, S. C. (2017). Microbial compositional changes in broiler chicken cecal contents from birds challenged with different *Salmonella* vaccine candidate strains. *Vaccine, 35*(24), 3204.

Patel, S., & McCormick, B. A. (2014). Mucosal inflammatory response and *Salmonella typhimurium* infection. *Frontiers in Immunology, 5*, 311.

Patterson, N. J., & Werling, D. (2013). TO con protection: TIR-domain containing proteins (Tcp) and innate immune evasion. *Veterinary Immunology and Immunopathology, 155*, 147–154.

Pomeroy, B. S. (1988). Fowl typhoid. In M. S. Hofstad, H. J. Barnes, B. W. Calneck, W. M. Reid, & H. W. Yoder (Eds.), *Diseases of poultry* (pp. 79–81). Ames, IA: Iowa State University Press.

Porter, R. E., & Holt, P. S. (1992). Effect of induced molting on the severity of intestinal lesions caused by *Salmonella enteritidis* infection in chickens. *Avian Diseases, 37*, 1009–1016.

Prejit, A., Agarwal, R. K., Porteen, K., Dubal, Z. B., Asha, K., Shweta, S., et al. (2013). Evaluation of recombinant outer membrane protein based vaccine against *Salmonella* Typhimurium in birds. *Biologicals, 41*, 162–168.

Raetz, C. R. H., & Whitfield, C. (2002). Lipopolysaccharide endotoxins. *Annual Review of Biochemistry, 71*, 635–700.

Revolledo, L., & Ferreira, A. J. P. (2012). Current perspectives in avian salmonellosis: Vaccines and immune mechanisms of protection. *Journal of Applied Poultry Research, 21*, 418–430.

Roberts, T., Wison, J., Guthrie, A., Cookson, K., Vancraeyrest, D., Schaeffer, J., et al. (2015). New issues and science in broiler chicken intestinal health: Intestinal microbial composition, shifts, and impacts. *World's Poultry Science Journal, 71*, 259–270.

Roof, M. B., & Kramer, T. T. (1989). Porcine neutrophil function in the presence of virulent and avirulent *Salmonella choleraesuis*. *Veterinary Immunology and Immunopathology, 23*, 365–376.

Rubinelli, P. M., Kim, S. A., Park, S. H., Baker, C. A., & Ricke, S. C. (2017). Growth characteristics of single and double Salmonella methionine auxotroph strains for potential vaccine use in poultry. *Frontiers in Veterinary Science, 4*, 103.

Rubinelli, P. M., Lee, S. I., Roto, S. M., Park, S. H., & Ricke, S. C. (2015). Regulated expression of virulence gene *mviN* provides protective immunity and colonization control of *Salmonella* in poultry. *Vaccine, 33*, 5365–5370.

Rychlik, I., Karasova, D., Sebkova, A., volf, J., Sisak, F., Havlickova, H., et al. (2009). Virulence potential of five major pathogenicity islands (SPI-1 to SPI-5) of *Salmonella enterica* serovar Enteritidis for chickens. *BMC Microbiology, 9*, 268.

Saliba, A. E., Li, L., Westermann, A. J., Appenzeller, S., Stapelis, D. A., Schulte, L. N., et al. (2016). Single cell RNA-seq ties macrophage polarization to growth rate of *Salmonella*. *Nature Microbiology, 2*, 16206.

Setta, A. M., Barrow, P. A., Kaiser, P., & Jones, M. A. (2012a). Early immune dynamics following infection with *Salmonella enterica* serovars Enteritidis, Infantis, Pullorum, and Gallinarum: Cytokine and chemokine gene expression profile and cellular changes in chicken cecal tonsils. *Comparative Immunology, Microbiology and Infectious Diseases, 35*, 397–410.

Setta, A. M., Barrow, P. A., Kaiser, P., & Jones, M. A. (2012b). Immune dynamics following infection of avian macrophages and epithelial cells with typhoid and non-typhoid *Salmonella enterica* serovars: Bacterial invasion and persistence, nitric oxide and oxygen production, differential gene expression, NF-kappaB signaling and cell cytotoxicity. *Veterinary Immunology and Immunopathology, 146*, 212–224.

Shanmugasundaram, R., Kogut, M. H., Arsenault, R. L., Swaggerty, S. L., Cole, K. Y., Reddish, M. J., et al. (2015). Effect of *Salmonella* infection on cecal tonsil regulatory T cell properties in chicken. *Poultry Science, 94*, 1828–1835.

Shapiro, H., Lutaty, A., & Ariel, A. (2011). Macrophages, meta-inflammation, and immunometabolism. *Scientific World Journal, 11*, 2509–2529.

Shin, S., & Brodsky, I. E. (2015). The inflammasome: Learning from bacterial evasion strategies. *Seminars in Immunology, 27*, 102–110.

Shirey, K. A., Cole, L. E., Keegan, A. D., & Vogel, S. N. (2008). *Francisella tularensis* live vaccine strain induces macrophage alternative activation as a survival mechanism. *Journal of Immunology, 181*, 4159–4167.

Si, W., Wang, X., Liu, H., Yu, S., Li, Z., Chen, L., et al. (2015). Physiology, pathogenicity, and immunogenicity of live, attenuated *Salmonella enterica* serovars Enteritidis mutants in chicks. *Microbial Pathogenesis, 83-84*, 6–11.

Sica, A., & Mantovani, A. (2012). Macrophage plasticity and polarization *in vivo veritas*. *The Journal of Clinical Investigation, 122*, 787–795.

Stabler, J., McCormick, T. C., Powell, K. C., & Kogut, M. H. (1993). Avian heterophils and monocytes: Phagocytic and bactericidal activities against *Salmonella enteritidis*. *Veterinary Immunology and Immunopathology, 38*, 293–305.

Stecher, B., Hapfelmeier, S., Miller, C., Kremer, M., Stallmach, T., & Hardt, W.-D. (2004). Flagella and chemotaxis are required for efficient induction of *Salmonella enterica* serovars Typhimurium colitis in streptomycin-pretreated mice. *Infection and Immunity, 72*, 4138–4150.

Steele-Mortimer, O. (2008). The *Salmonella*-containing vacuole: Moving with the times. *Current Opinion in Microbiology, 11*, 38–45.

Storek, K. M., & Monack, D. M. (2015). Bacterial recognition pathways that lead to inflammasome activation. *Immunological Reviews, 265*, 112–129.

Takeuchi, A., & Sprinz, H. (1967). Electron microscope studies of experimental *Salmonella* infections in the pre-conditioned guinea pig. II. Response of the intestinal mucosa to the invasion by *Salmonella typhimurium*. *The American Journal of Pathology, 51*, 137–146.

Thompson, J. A., Liu, M., Helaine, S., & Holden, D. W. (2011). Contribution of the PhoP/PhoQ regulon in survival and relocation of *Salmonella enterica* serovars Typhimurium in macrophages. *Microbiology, 157*, 2084–2093.

Thornbrough, J. M., & Worley, M. J. (2012). A naturally occurring single nucleotide polymorphism in the *Salmonella* SPI-2 type III effector srfH/sseI controls early extraintestinal dissemination. *PLoS One, 7*, e45245.

Tobar, J. A., Carreno, L. J., Bueno, S. M., Gonzalez, P. A., Mora, J. E., Quezada, S. A., et al. (2006). Virulent *Salmonella enterica* serovars Typhimurium evades adaptive immunity by preventing dendritic cells from activating T cells. *Infection and Immunity, 74*, 6438–6448.

Van der Heijden, J., Bosman, E. S., Reynolds, L. A., & Finlay, B. B. (2015). Direct measurement of oxidative and nitrosative stress dynamics in Salmonella inside macrophages. *PNAS, 112*, 560–565.

Van Dissel, J. T., Stikkelbroeck, J. J. M., Sluiter, W., Leijh, P. C. J., & Van Furth, R. (1986). Differences in initial rate of intracellular killing of *Salmonella typhimurium* by granulocytes

of *Salmonella*-susceptible C57BL/10 mice and *Salmonella*-resistant CBA mice. *Journal of Immunology, 136*, 1074–1080.

Van Immerseel, F., De Buck, J., De Smrt, I., Mast, J., Haesebrouck, F., & Ducatelle, R. (2002). The effect of vaccination with a *Salmonella* Enteritidis *aroA* mutant on early cellular responses in cecal lamina propria of newly-hatched chickens. *Vaccine, 20*, 3034–3041.

Van Immerseel, F., De Zutter, L., Hout, K., Passmans, F., Haesebrouck, F., & Ducatelle, R. (2009). Strategies to control *Salmonella* in the broiler production chain. *World's Poultry Science Journal, 65*, 367–391.

Vazquez-Torres, A., Jones-Carson, J., Baumler, A. J., Falkow, S., Valdivia, R., Brown, W., et al. (2000). *Salmonella* pathogenicity island-2-dependent evasion of the phagocyte NADPH oxidase. *Science, 287*, 1655–1658.

Waterman, S., & Holden, D. W. (2003). Functions and effectors of the *Salmonella* pathogenicity island-2 type III secretion system. *Cellular Microbiology, 5*, 501–511.

Wick, M. J. (2011). Innate immune control of *Salmonella enterica* serovars Typhimurium: Mechanisms contributing to containing systemic *Salmonella* infection. *Journal of Innate Immunity, 3*, 543–549.

Wideman, R. F., Hamal, K. R., Stark, J. M., Blankenship, J., Lester, H., Mitchell, K. N., et al. (2012). A wire-floor model for inducing lameness in broilers: Evaluation of probiotics as a prophylactic treatment. *Poultry Science, 91*, 870–883.

Wigley, P. (2014). *Salmonella enterica* in the chicken: How it has helped our understanding of immunology in a non-biomedical model species. *Frontiers in Immunology, 5*, 482.

Wigley, P., & Barrow, P. (2017). *Salmonella* in preharvest chickens: Current understanding and approaches to control. In S. C. Ricke & R. K. Gast (Eds.), *Producing safe eggs: Microbial ecology of Salmonella* (pp. 139–159). New York: Academic Press.

Wigley, P., Hulme, S., Powers, C., Beal, R., Smith, A., & Barrow, P. (2005). Oral infection with the *Salmonella enterica* serovars Gallinarum 9R attenuated live vaccine as a model to characterize immunity to fowl typhoid in the chicken. *BMC Veterinary Research, 1*, 2.

Wigley, P., Hulme, S., Rothwell, L., Bumstead, N., Kaiser, P., & Barrow, P. (2006). Macrophages isolated from chickens genetically resistant tor susceptible to systemic salmonellosis show magnitudinal and temporal differential expression of cytokines and chemokines following *Salmonella enterica* challenge. *Infection and Immunity, 74*, 1425–1430.

Withange, G. S., Kaiser, P., Wigley, P., Powers, C., Mastroeni, P., Brooks, H., et al. (2004). Rapid expression of chemokines and pro-inflammatory cytokines in newly hatched chickens infected with *Salmonella enterica* serovar Typhimurium. *Infection and Immunity, 72*, 2152–2159.

Withange, G. S., Mastroeni, P., Brooks, H., Maskell, D., & McConnell, I. (2005a). Oxidative and nitrosative responses of the chicken macrophage cell line MQ-NCSU to experimental *Salmonella* infection. *British Poultry Science, 46*, 261–267.

Withange, G. S., Wigley, P., Kaiser, P., Mastroeni, P., Brooks, H., Powers, C., et al. (2005b). Cytokine and chemokine responses associated with clearance of a primary *Salmonella enterica* serovar Typhimurium infection in the chicken and in protective immunity to rechallenge. *Infection and Immunity, 73*, 5173–5182.

Worley, M. J., Nieman, G. S., Geddes, K., & Hefron, F. (2006). *Salmonella typhimurium* disseminates within its host by manipulating the motility of the infected cells. *PNAS, 103*, 17915–17920.

Wu, H., Jones, R. M., & Neish, A. S. (2012). The *Salmonella* effector AvrA mediates bacterial intracellular survival during infection in vivo. *Cellular Microbiology, 14*, 23–29.

Wynosky-Dolfi, M. A., Snyder, A. G., Philip, N. H., Doonan, P. J., Poffenberger, M. C., Avizonis, D., et al. (2014). Oxidative metabolism enables *Salmonella* evasion of the NLRP3 inflammasome. *The Journal of Experimental Medicine, 211*, 653–668.

Xavier, M. N., Winter, M. G., Specs, A. M., den Hartigh, A. D., Nguyen, K., Roux, C. M., et al. (2013). PPARɣ-mediated increase in glucose availability sustains chronic *Brucella abortus* infection in alternatively activated macrophages. *Cell Host & Microbe, 14*, 159–170.

Ye, Z., Petrot, E. O., Boone, D., Claud, E. C., & Sun, J. (2007). *Salmonella* effector AvrA regulation of colonic epithelial cell inflammation by debiquitination. *The American Journal of Pathology, 71*, 882–892.

Yi, J., Kloeker, S., Jensen, C. C., Bockholt, S., Honda, H., Hirai, H., et al. (2002). Members of the Zyxin family of LIM proteins interact with members of the p130Cas family of signal transducers. *The Journal of Biological Chemistry, 277*, 9580–9589.

Yu, L. C.-H., Wang, J.-T., Wei, S.-C., & Ni, Y.-H. (2012). Host-microbial interactions and regulation of intestinal epithelial barrier function: From physiology to pathology. *World Journal of Gastrointestinal Pathophysiology, 3*, 27–43.

Zhang, S., Lillehoj, H. S., Kim, C. H., Keeler, C. L., Babu, U., & Zhang, M. Z. (2008). Transcriptional response of chicken macrophages to *Salmonella enterica* serovars Enteritidis infection. *Developmental Biology, 132*, 141–151.

Chapter 9
Advances in Vaccines for Controlling *Campylobacter* in Poultry

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9.1 Introduction: Current Prevalence of *Campylobacter* in Poultry Industry

Campylobacter jejuni is the most common cause of foodborne bacterial gastroenteritis worldwide (World Health Organization (WHO) 2015). Chicken is the most frequent source of human *Campylobacter* infection, and control of infection in poultry production is a public health priority (Umaraw et al. 2015). Effective vaccination, which has proved successful for the control of *Salmonella enterica* in chicken and egg production, offers considerable long-term potential in controlling *C. jejuni*, but vaccine development has been hampered by a relatively poor understanding of the infection biology and colonization of *C. jejuni* and in particular the immune response to the bacterium in chickens (Hermans et al. 2011).

Campylobacter species, primarily *C. jejuni* and *C. coli*, are highly prevalent in poultry production systems, such as broilers, layers, turkeys, and ducks (Sahin et al. 2012; Zhang and Sahin 2013; Gormley et al. 2014; Kashoma et al. 2014). Prevalence of *Campylobacter*-positive poultry flocks are generally high but vary by regions, seasons, and production types (conventional, free-range, organic, etc.), with reported *Campylobacter*-positive flocks ranging from 2% to 100% (Jore et al. 2010; Ansari-Lari et al. 2011; Berghaus et al. 2013; Kalupahana et al. 2013; Golz et al. 2014). It appears that the prevalence of *Campylobacter* is lower in Scandinavian countries than in other European countries, North America, and developing countries. Seasonal variations have been observed in the prevalence of *Campylobacter* flocks,

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with a peak in summer and autumn months (Bouwknegt et al. 2004; Barrios et al. 2006). A high prevalence of *Campylobacter* in warm months may be due to an increased fly population resulting in fly-mediated transmission (Hald et al. 2004; Bahrndorff et al. 2013). *Campylobacter* is more prevalent in organic and free-range flocks than in conventional production (Ring et al. 2005; Wittwer et al. 2005; Luangtongkum et al. 2006; Van Overbeke et al. 2006; Allen et al. 2011). Free access to the outside environments and longer life span are main reasons contributing to the increased prevalence rates of *Campylobacter* in organic and free-range productions (Zhang and Sahin 2013).

9.2 Transmission and Control of *Campylobacter* in Poultry

Birds are naturally infected with *Campylobacter* via the fecal-oral route, after which the organism establishes itself in the intestinal tract with the main site of colonization being the ceca and colon and to a lesser extent the small intestines, liver, and other organs (Sahin et al. 2002; Hermans et al. 2012; Chaloner et al. 2014). Although young birds may develop clinical disease (e.g., diarrhea and weight loss), as shown in some experimental infections with *Campylobacter* (Lam et al. 1992; Humphrey et al. 2014), the vast majority of studies revealed a commensal nature of the organism in poultry, with no clinical signs of disease (Newell and Wagenaar 2000; Sahin et al. 2003; Knudsen et al. 2006; Zhang and Sahin 2013). A large number of *Campylobacter* (up to 10^9 CFU/g feces) can be recovered in ceca and excreted in feces for a prolonged period (e.g., at least until the slaughter age) following the establishment of organisms in the intestinal tract after both natural and experimental infections (Elvers et al. 2011; Hermans et al. 2012). Under the conditions of commercial production, chicken flocks can be colonized by single or multiple species and genotypes of *Campylobacter* even during a single rearing cycle (Bull et al. 2006; Elvers et al. 2011; Herman et al. 2003; Rivoal et al. 2005; Stern and Pretanik 2006; Wittwer et al. 2005), which has also been reproduced in experimental infections (Chaloner et al. 2014).

The factors commonly associated with *Campylobacter* colonization in broiler flocks include lack of overall biosecurity on farms (Hansson et al. 2007, 2010; Allen et al. 2008; Patriarchi et al. 2011), presence of other animals in close proximity to poultry houses (Bouwknegt et al. 2004; Ellis-Iversen et al. 2012; Jonsson et al. 2012; Sommer et al. 2013), age and number of poultry houses on a farm, slaughter age, size of flocks, the practice of partial depopulation (thinning), seasonal and climate changes, use of ventilators, fly population (and lack of fly screens), use of old litter, farm equipment, transport vehicles, and farm workers (Kassem et al. 2010). Rarely, feed, fresh litter, and water may act as sources for the initial introduction of *Campylobacter* into poultry flocks, although they can be contaminated by the organism in poultry houses, where the birds are colonized and thus can facilitate the spread of *Campylobacter* within production facilities (Line 2002; Zimmer et al. 2003; Jacobs-Reitsma et al. 2008).

In flocks, *Campylobacter* colonization naturally occurs by horizontal transmission from the environment in 2- or 3-week-old chickens due to the availability of protective maternal antibodies in chicken sera in the first weeks post hatching (Sahin et al. 2003; Cawthraw and Newell 2010). During the first weeks of life, maternal antibody levels progressively decrease until fully degraded at the end of the third week (Cawthraw and Newell 2010). *Campylobacter* infection spreads very quickly in the flock by horizontal transmission from one bird to another after first infection. van Gerwe et al. (2010) estimated a transmission rate of 2.37 new infections per infected bird per day, confirming experimental results (Stern et al. 2001). This means that *Campylobacter* prevalence increases from one infected bird to 95% of a whole flock of 20,000 broilers within a week. This rapid *Campylobacter* transmission could be explained by high fecal shedding, contamination of drinking water and litter, and coprophagic behavior of chickens. High flock size, environmental water supplies, litter, insects, wild birds, rodents, fecal contact, personnel, and other animals may increase the risk of colonization and dissemination (Line 2002; Zimmer et al. 2003; Adkin et al. 2006; Jacobs-Reitsma et al. 2008; Horrocks et al. 2009; Silva et al. 2011; Sahin et al. 2015).

There is continuing debate about the relative contribution of vertical transmission of *Campylobacter* spp. from breeding flocks. Notably, many studies concluded that vertical transmission from breeder flocks via eggs was not a major source of *Campylobacter* infection in broiler houses (Barrios et al. 2006; Callicott et al. 2006; Patriarchi et al. 2011), although some controversy still exists (Cox et al. 2012). The circumstantial evidence for the possible spread of *Campylobacter* by vertical transmission was proved in several studies. In these studies, organism was isolated from the outer and inner shell surface of eggs laid by *Campylobacter*-positive commercial layers or broiler breeders (Shane et al. 1986; Shanker et al. 1986), from the reproductive tract of hens (Buhr et al. 2002; Hiett et al. 2002), and from semen of broiler breeder roosters (Cox et al. 2002). However, lack of *Campylobacter* colonization during the first weeks of life of broilers, those hatched from eggs originated from breeder flocks infected with *Campylobacter* under natural farms settings, argues against the importance of vertical transmission (van de Giessen et al. 1992, 1998; Barrios et al. 2006; Bull et al. 2006; Callicott et al. 2006).

9.3 Control Approaches for *Campylobacter* in Poultry Farms

On-farm preventive measures against *Campylobacter* in poultry have been comprehensively reviewed (Wagenaar et al. 2008; de Zoete et al. 2007; Connerton et al. 2008). Several strategies have been proposed for the control of *Campylobacter* on the poultry farm including (1) reduction of environmental exposure (biosecurity measures), (2) an increase in poultry host resistance to reduce *Campylobacter* carriage in the gut (e.g., competitive exclusion, vaccination, and host genetics selection), and (3) the use of antimicrobial alternatives to reduce and even eliminate *Campylobacter* from colonized chickens (e.g., bacteriophage therapy and bacteriocin treatment).

9.3.1 Good Hygienic Practices and Biosecurity

Implementing strict biosecurity and good hygiene measures helps to prevent *Campylobacter* contamination of broiler houses from the outside environment. These practices include washing hands before engaging the flocks, designating separate boots and personal gear for different broiler houses, deploying footbaths for disinfection, limiting access to the flocks to only essential personnel, training workers in best hygiene practices, controlling pests such as rodents and insects, thorough decontamination of drinking water delivery systems, and maintaining the physical structure of broiler houses (Hermans et al. 2011; Sahin et al. 2015). However, even the most stringent biosecurity measures do not always have a consistent and predictable effect on controlling *Campylobacter*, and their effectiveness in controlling flock prevalence is difficult to assess under commercial settings (Arsenault et al. 2007; Nather et al. 2009; Ridley et al. 2011; Sahin et al. 2015). In addition, stringent biosecurity measures are cost prohibitive and hard to maintain, and their effectiveness varies with production systems (Sahin et al. 2002, 2015). Additionally, the differences in production practices between countries affect the success of certain biosecurity and hygiene approaches, which poses a significant challenge for evaluating and adopting universal control protocols (Zhang and Sahin 2013).

9.3.2 Treatment of Drinking Water

Studies have shown that poor-quality water (untreated water from wells) may increase the transmission of *Campylobacter* in animals (Lyngstad et al. 2008; Sparks 2009). Therefore, the microbiological quality of drinking water should be routinely monitored and could be improved by techniques such as filtration, chlorination, ozonation, and UV rays. Acidification of drinking water has also been reported to decrease the risk of *Campylobacter* colonization in broiler flocks (Allain et al. 2014). Addition of 0.44% (vol/vol) lactic acid in drinking water prior to slaughter was found to reduce the level of contamination of carcasses with *Campylobacter* (Byrd et al. 2001). Furthermore, Hilmarsson et al. (2006) showed that addition of glycerol monocaprate (monocaprin) during the last 3 days before slaughter reduced the number of *C. jejuni* in feces of chickens naturally or experimentally infected. Moreover, water treatment such as chlorination has been reported to reduce *Campylobacter* counts in cloacal samples, but did not affect transmission between broilers and overall prevalence (Stern et al. 2002).

The impact of aforementioned interventions on *Campylobacter* infection is uncertain, but it has been pointed out that in the absence of interventions, the *Campylobacter* prevalence may be worst in poultry flock and processed chickens (Gibbens et al. 2001; Mohyla et al. 2007).

9.3.3 Use of Bacteriophages

Bacteriophages were discovered at the beginning of the nineteenth century, and they are natural bacterial killers ubiquitous in the environment. The use of phages to control animal diseases has given promising results and led researchers to consider *Campylobacter* bacteriophages as a tool to combat chicken colonization (Loc Carrillo et al. 2005; Wagenaar et al. 2005; El-Shibiny et al. 2009; Carvalho et al. 2010; Fischer et al. 2013; Kittler et al. 2013). Bacteriophages can be administered either individually (Loc Carrillo et al. 2005; Wagenaar et al. 2005; El-Shibiny et al. 2009) or as cocktail (Wagenaar et al. 2005; Carvalho et al. 2010; Fischer et al. 2013; Kittler et al. 2013). Due to their host-specific nature, based on interactions between phage tail proteins and bacterial receptors, phage treatment could be the answer to the emergence of antibiotic-resistant bacterial strains. As the avian gut represents the main reservoir of *Campylobacter*, most studies have used phages isolated from the chicken gastrointestinal tract. Janez and Loc-Carrillo (2013) have described the isolation and characterization of *Campylobacter* phages. Phages replicate only in the target bacterial cell, and due to their host specificity, bacteriophages applied to combat *Campylobacter* do not alter normal gut flora. Although individual phage administration could be effective in decreasing *Campylobacter* counts in chickens, phage and bacterial strains determine the treatment's success. Therefore, it may be more effective to administer several phages in combination to overcome host specificity.

9.3.4 Use of Probiotics and Prebiotics

Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit to the host (WHO 2001). Probiotics are generally nonpathogenic bacteria and viable microorganisms that can confer beneficial effects on the host by maintaining gut microbial balance and homeostasis and facilitating mucosal repair in the gastrointestinal tract (Behnsen et al. 2013; Mohan 2015; Sherman et al. 2005). Probiotics provide an effective means of preventing or reducing the incidence of *Campylobacter* infection in animal hosts in an antibiotic-free manner (Fanelli et al. 2015; Kemmett 2015; Saint-Cyr et al. 2016). Several studies have focused on preventing *Campylobacter* colonization in broiler chickens at the primary production stage, typically by competitive exclusion of the pathogen by the probiotics (Bratz et al. 2015; Stef 2016; Thomrongsuwannakij et al. 2016; Helmy et al. 2017). However, probiotics have large discrepancies in terms of intestinal *Campylobacter* load reduction (Meunier et al. 2015) and the mechanisms of competitive exclusions including the occupation of adhesions sites and receptors, secretion of antimicrobial substances, and competition for essential nutrients (Bratz et al. 2015).

9.3.5 Use of Bacteriocins

Bacteriocins are designated as the antimicrobial peptides (AMPs) produced by bacteria with narrow or broad host ranges (Hechard and Sahl 2002; Riley and Wertz 2002; Cotter et al. 2005; Sit and Vederas 2008). Bacteriocins have been used to control *Campylobacter* colonization in poultry (Table 9.1). Several types of bacteriocins have been produced and tested for their efficacy in reduction of *Campylobacter* colonization in poultry. The commonly used bacteriocins include bacteriocin OR-7 isolated from *Lactobacillus salivarius* strain NRRL B-30514 (Stern et al. 2006), bacteriocin from *Paenibacillus polymyxa* strain NRRL B-30509 (Stern et al. 2005), L-1077 bacteriocin isolated from another *L. salivarius* strain (Svetoch et al. 2011),

Table 9.1 Overview of bacteriocins that reduced *Campylobacter* colonization in poultry

Bacteriocin-producing bacteria				
Strain	Species	Bacteriocin name	Effect[a]	References
NRRL B-30509	*Paenibacillus polymyxa*	SRCAM 602	• ND (4.6–6.3 log reduction in 10-day-old chickens) • ND (>4 log reduction in 13-day-old turkeys)	Stern et al. (2005) Svetoch et al. (2005) Cole et al. (2006)
NRRL B-30514	*Lactobacillus salivarius*	OR-7	• >6 log reduction in 10-day-old chickens • ND (>4 log reduction in 13-day-old turkeys)	Stern et al. (2006) Cole et al. (2006)
NRRL B-30745	*Enterococcus durans/faecium/hirae*	E-760	• ND (>6.6 log reduction in 10-day-old chickens with dose as low as 31.2 mg/kg feed) • ND (2.2–5.0 log reduction in 42-day-old chickens with dose of 125 mg/kg feed)	Line et al. (2008)
NRRL B-30746	*Enterococcus faecium*	E 50–52	• ND (>6.4 log reduction in 15-day-old chickens with dose as low as 31.2 mg/kg feed) • >5.3 log reduction with 1-day treatment of 35- to 41-day-old broilers (12.5 mg of E 50–52 = liter of drinking water)	Svetoch et al. (2008)

[a]ND, no *Campylobacter* was detected in all birds after bacteriocin treatment with minimum detection of 100 CFU/g cecal contents. Unless specifically clarified, treated birds were provided specific bacteriocin at a dose of 250 mg/kg feed for 3 consecutive days. The bacteriocins were mixed with polyvinylpyrrolidone powder to produce microencapsulated bacteriocins, which were used to make a medicated feed

and SMXD51 bacteriocin produced by a *L. salivarius* strain (Messaoudi et al. 2012). Two other bacteriocins produced by *Enterococcus* species, which are not lactic acid bacteria, have also been identified: E-760, from the NRRL B-30745 strain, and E 50–52, from the *Enterococcus faecium* NRRL B-30746 strain (Line et al. 2008; Svetoch et al. 2008). The above studies have suggested that the application of purified anti-*Campylobacter* bacteriocins is generally a more efficient way of decreasing *Campylobacter* in chickens' intestine than probiotic strains. This could be due to the fact that bacteriocins which are released by administrated probiotic strains are likely to be at a lower concentration compared to directly administrated bacteriocins.

9.4 The Use of Vaccine in Controlling *Campylobacter* in Poultry

The challenges for an effective vaccine against *Campylobacter* in poultry are significant. There is a correlation between increasing levels of *Campylobacter* antibodies and reducing levels of *C. jejuni* colonization in poultry (Lin 2009). Vaccination is commonly recognized as the most effective strategy to prevent human infectious diseases caused by bacterial and viral pathogens. Several human intestinal diseases, including campylobacteriosis, are zoonoses, acquired by contact with, or by consumption of, contaminated animal products (Kobierecka et al. 2016). Various vaccination strategies are currently being developed to combat *C. jejuni* in poultry. It is known that the level of maternal antibodies in chicks remains high for 3 to 4 days after hatching and then gradually decreases to undetectable levels by 2–3 weeks of age (Sahin et al. 2002). Researchers have also found that presence of anti-*C. jejuni* antibodies in birds prior to exposure results in a decrease in the bacterial ability to colonize chickens (Rice et al. 1997; Wyszynska et al. 2004).

9.5 Vaccination Approaches in Controlling *Campylobacter* in Poultry

Since *Campylobacter* is a major public health issue in developed countries, poultry vaccination remains one of the best strategies to control campylobacteriosis incidence. To date, many vaccination studies have been conducted using various strategies, including whole-cell or subunit vaccines and microorganism-vectored vaccines (Table 9.2).

(1) *Whole-cell vaccines (WCV)*. Whole-cell vaccines (WCV) were the first to be investigated and consist of administering killed or attenuated bacteria devoid of virulent and/or colonizing abilities. Rice et al. (1997) investigated the efficacy of formalin-inactivated *C. jejuni* strain F1BCB in chickens and reported a

Table 9.2 Vaccination experiments against *Campylobacter jejuni* in poultry

Vaccine source	Vaccine strain	Challenge strain	*Campylobacter* colonization results	References
Whole-cell vaccines				
C. jejuni formalin-inactivated	*C. jejuni*, F1BCB	*C. jejuni*, F1BCB	Colonization reduced by 93% on day 46	Rice et al. (1997)
C. jejuni, formol-inactivated	*C. jejuni*, UO6	*C. jejuni*, UO1LIO 6	Lower *Campylobacter* excretion after vaccination in week 7 and after homologous challenge	Glünder et al. (1997)
WC + flagellin	*C. jejuni*, isolate #V2	*C. jejuni*, isolate #V2 Exposure to infected birds	Significant cecal reduction for birds immunized intraperitoneal twice	Widders et al. (1998)
Subunit vaccine				
Flagellin DNA	*C. jejuni*, ALM-80	*C jejuni*, ALM-80 5×10^7 CFU	Colonization reduction in cecum and large intestine and total clearance in small intestine on day 60	Huang et al. (2010)
FlaA/CadF/FlpA/ CmeC protein or CadF-FlaA-FlpA fusion protein	*C. jejuni* F38011	*C. jejuni* F38011 2×10^8 CFU	Significant cecal reduction on day 27	Neal-McKinney et al. (2014)
CjaA protein	*C. jejuni*, M1	*C. jejuni*, M1 10^7 CFU	Significant cecal reduction on day 28	Buckley et al. (2010)
N-glycan protein	Live or inactivated *E. coli* strain expressing the LPS core-N-glycan	*C. jejuni*, 81–176 10^2 or 10^6 CFU	Significant cecal reduction on day 35	Nothaft et al. (2016)
N-glycan protein with probiotics (*A. mobilis* DSM 15930 or *L. reuteri* CSF8)	Live or inactivated *E. coli* strain expressing the LPS core-N-glycan	*C. jejuni*, 81–176 10^6 CFU	Significant cecal reduction on day 35	Nothaft et al. (2017)
Antigens				
Outer membrane proteins	*C. jejuni*, 81–176	*C. jejuni*, 81–176 2×10^7 CFU	• Oral: slight cecal reduction • Sc: significant reduction in cecal and cloacal loads	Annamalai et al. (2013)
CjaA protein via *Salmonella enterica* strain ×3987	*C. jejuni*, 72Dz/92	*C. jejuni*, pUOA18 2×10^8 CFU	Cecal reduction on day 12 post challenge	Wyszynska et al. (2004)

(continued)

Table 9.2 (continued)

Vaccine source	Vaccine strain	Challenge strain	*Campylobacter* colonization results	References
CjaA protein fused to the tetanus toxin fragment C, via *Salmonella typhimurium* 4/74 nal® ΔaroA	*C. jejuni*, MI	*C. jejuni*, MI 10^7 CFU	Significant cecal reduction from day 21 post challenge	Buckley et al. (2010)
CjaA/Peb1A protein fused to the tetanus toxin fragment via *Salmonella typhimurium* 4/74 nal® ΔaroA	*C. jejuni*, MI	*C. jejuni*, MI 107 CFU	Cecal reduction of 1.64log10 CFU g^{-1} in vaccinated group compared to control group	Buckley et al. (2010)

significant reduction of *Campylobacter* load from 16 to 93% in the vaccinated groups compared to unvaccinated control group. Furthermore, IgA titers in serum or bile were generally higher in vaccinated birds than in the control group, with more immune-responding birds. However, the heat-labile toxin (LT) adjuvant did not impact vaccine efficacy. Contrary to these findings, other groups did not obtain consistent results. For example, Glünder et al. (1997) showed that although specific antibodies were generated in chicken serum after subcutaneous immunization of formol-inactivated *C. jejuni* and complete Freund's adjuvant, vaccination had little effect on intestinal colonization after a homologous challenge and none at all after a heterologous inoculation. Vaccination of chicks with four viable but non-colonizing *C. jejuni* strains did not give protective immunity despite chicks' immunological competence, and all the birds were colonized like the positive control group, regardless of the tested experimental conditions (Ziprin et al. 2002). Widders et al. 1998 observed a significant reduction in cecal *C. jejuni* loads when birds were immunized twice intraperitoneally with WCV combined with purified flagellin. In another study Noor et al. (1995) showed that chicks immunized in ovo and boosted orally after hatching with WCV produced strong immune response since IgY, IgA, and IgM were detected in serum and IgA in intestinal contents and bile. The oral booster led to a higher increase in secreted IgA levels in the bile and intestines. These results indicate the development of an immune response before hatching, but the protective potential of this vaccine was not evaluated (Noor et al. 1995).

(2) *Subunit vaccines*. The first subunit vaccine in chickens was based on the immunodominant antigen of *Campylobacter,* flagellin. This is the main component of bacterial flagella, which plays a crucial role in bacterial colonization. Subunit flagellin vaccination gave inconsistent results from one study to another. Widders et al. (1998) used purified native flagellin for subunit vaccination. Although this led to the development of a specific humoral immune response at both systemic and mucosal level, no significant reduction in cecal *Campylobacter*

loads was observed after the challenge. However, when flagellin protein fused to the B subunit of the *E. coli* labile toxin, and orally administered twice at the higher dose of 1 mg, flagellin induced specific antibodies in more than two-thirds of the vaccinated birds. The vaccinated birds had lower *C. jejuni* counts than the control group after an oral challenge (Khoury and Meinersmann 1995). More recently, Neal-McKinney et al. (2014) demonstrated that birds vaccinated with flagellin combined with the Montanide adjuvant showed a 3 log10 CFU g^{-1} intestinal reduction compared to the control group, in addition to a higher specific sera reactivity. Huang et al. (2010) tested flagellin vaccination using DNA by the intranasal route with chitosan nanoparticles in which pCAGGS-flaA, a DNA plasmid used as the flagellin A vector, was incorporated. After the second and third immunizations, significantly higher specific antibody titers were detected for both serum IgY and intestine mucosal IgA compared to the control groups, along with a decrease in bacterial loads of 2–3 and 2 log10 CFU g^{-1} in the large intestine and cecum, respectively, after an oral challenge. Interestingly, *C. jejuni* was absent from the small intestine at the end of the study, confirming the immunization potential of the *Campylobacter* flagellin.

However, despite promising results, flagellin cannot be used as an antigen for large-scale poultry vaccination for several reasons: (1) there are differences in flagellin between *Campylobacter* strains and a lack of cross protection against various strains susceptible to colonize broilers, (2) many anti-flagellin antibodies are directed against non-surface-exposed epitopes and consequently do not neutralize the bacterium during infection (Widders et al. 1998), and (3) some antibodies recognize glycosylated patterns with variable phases, allowing *Campylobacter* to evade the immune system of host, and this difference is due to varying amount and nature of these residues.

More antigens were also tested in subunit vaccine experiments in chickens. The CjaA protein, known as the binding protein component of an ABC transporter system (Muller et al. 2005), was inoculated on day 1 or day 15 post hatching. In both experimental groups, significantly higher specific IgY titers were detected than in the control group and were the same for both inoculation periods. Cecal *Campylobacter* loads were also similar in both groups on day 21 post challenge, although slightly greater on day 28 post challenge when birds were first vaccinated on day 15 and always significantly lower than in the infected control group, indicating the immunization potential of the CjaA protein (Buckley et al. 2010). Another study, using outer membrane proteins (OMP) encapsulated in poly(lactide-co-glycolide) (PLGA) nanoparticles, was conducted to decrease *C. jejuni* colonization in chickens. When OMP-PLGA nanoparticle was administered orally, there was no significant reduction in *Campylobacter* load regardless of the tested doses. However, subcutaneous vaccination was found more effective, where intestinal colonization level of *Campylobacter* dropped below the detection limit compared to the control group, and was accompanied by the development of a strong immune response (Annamalai et al. 2013). Another study investigating the role of *dps* gene in biofilm formation revealed its involvement in *C. jejuni* colonization in chickens and

suggested it as a potential vaccine antigen. However, recombinant Dps subunit vaccination subcutaneously did not protect chickens from *C. jejuni* colonization after a challenge (Theoret et al. 2012). More recently, several proteins such as CadF, FlpA, and CmeC, having a role in *Campylobacter* adherence during poultry colonization, were tested as antigens in subunit vaccination experiments. Using the Montanide adjuvant, all these proteins induced an increase in sera reactivity of vaccinated birds. Although cecal reductions were not significant for both CadF- and CmeC-vaccinated groups, FlpA immunization significantly reduced cecal load by about 3 log10 CFU g^{-1}. In addition, vaccination with the fused CadF-FlaA-FlpA protein and a mixture of the three full-length individual proteins as a booster led to a significant intestinal decrease of about 3 log10 CFU g^{-1} in *C. jejuni* (Neal-McKinney et al. 2014). Recently, Nothaft et al. (2016) used a novel approach to create an effective chicken vaccine against *C. jejuni*. Carbohydrates are class of biomolecules that have been successfully used for the generation of human glycoconjugate vaccines but are currently not commercially available for animals (Jones 2005). *C. jejuni* is rich in surface carbohydrates, including O- and N-linked glycoproteins, capsular polysaccharides (CPS), and lipooligosaccharides (LOS) (Szymanski et al. 2003; Nothaft et al. 2012). However, high serological diversity in CPS and O-linked glycoproteins and LOS limit the use of these carbohydrates as potential antigen. But glycoconjugate vaccines combining the conserved *C. jejuni* N-glycan with a protein carrier, GlycoTag, or fused to *Escherichia coli* lipopolysaccharide-core are promising antigen candidates for chickens. Vaccination of chickens with the protein-based or *E. coli*-vectored glycoconjugate vaccine displayed up to 10-log reduction in *C. jejuni* colonization and induced N-glycan-specific IgY responses (Nothaft et al. 2016). A recent study by Nothaft et al. (2017) suggested that co-administration of *C. jejuni* N-glycan-based vaccine with probiotics potentiates the vaccine efficacy. These researchers demonstrated that co-administration of the live vaccine with commonly colonizing probiotics of chicken gut (*Anaerosporobacter mobilis* or *Lactobacillus reuteri*) resulted in an increased vaccine efficacy, antibody response, and weight gain in broilers. All these studies revealed the potential immunization power of certain *Campylobacter* antigens and suggested the need for thorough investigations to confirm their vaccine features.

(3) Antigens vectored by microorganisms. In recent years, another method of vaccine delivery has been studied. This consists of delivering antigens using microorganisms harboring plasmids with the DNA of interest. For *Campylobacter* antigens, attenuated *Salmonella* strains have been widely used as a vector with the CjaA protein as the main focus of study. Although the use of *Campylobacter* protective antigens in attenuated *Salmonella* strains seems promising, the results are inconsistent between studies, and there are concerns regarding immunization schemes used and long-term efficacy of this approach. Wyszynska et al. (2004) showed an increase in specific anti-*Campylobacter* response for both IgY in serum and intestinal IgA compared to unvaccinated group, along with more than 6 log10 CFU g^{-1} decrease in cecal *Campylobacter* load on day 12 post challenge. In contrast, Laniewski et al. (2014) did not find any significant

reduction in cecal load, although they showed development of a humoral immune response and an increase in B-cell population in the cecal tonsils of the vaccinated group. In addition, Buckley et al. (2010) observed the development of a specific immune response and the decrease by approximately 1.4 log10 CFU g^{-1} of the intestinal *Campylobacter* count after vaccination with *Salmonella*-vectored CjaA- and Peb1A-based vaccines. Similar results were also obtained after a single vaccination with an attenuated *Salmonella*-vectored CjaA protein, followed by an oral challenge 3 weeks later (Layton et al. 2011). It was also shown that specific immune response development was not necessarily correlated with the decrease in intestinal level of *Campylobacter*. Taken together, results from these experiments suggest that the choice of strains is critical in determining the vaccination's success.

Other antigens vectored by attenuated *Salmonella* strains have been investigated to decrease *Campylobacter* colonization in poultry. For example, Layton et al. (2011) showed that a single vaccination of chickens on the day of hatching, ACE393-vectored antigen, encoding a probable periplasmic protein, led to significantly higher IgY levels than control groups and to an approximately nonsignificant 1 log10 CFU g^{-1} *Campylobacter* reduction in the ileum after an oral challenge on day 21. With the same immunization scheme, Omp18-/CjaD-vectored antigen yielded more promising results with significantly higher specific serum IgY and mucosal IgA titers, along with a significant drop in intestinal counts below the detection limit. The later results were confirmed in repeated experiments (Layton et al. 2011). Similarly, Buckley et al. (2010) showed that after two vaccinations, Peb1A antigen significantly reduced the cecal load of *C. jejuni* by 1.6 log10 CFU g^{-1} when fused to the tetanus toxin and *Salmonella*-vectored, whereas no decreases were observed for GlnH and ChuA. Furthermore, Theoret et al. (2012) showed that the Dps (DNA-binding protein from starved cells) are good antigens to reduce cecal *Campylobacter* colonization in broiler chicks when delivered orally through *Salmonella* vaccine vector.

9.6 Conclusion

Campylobacteriosis is the most prevalent bacterial foodborne gastroenteritis affecting humans worldwide. Poultry constitutes the main reservoir of *Campylobacter*, substantial quantities of which are found in the intestines following rapid colonization. Since *Campylobacter* infection of chickens primarily occurs at the farm level, as primary production is a crucial step in *Campylobacter* poultry contamination, controlling the colonization at this level could improve food safety at the subsequent links along the food chain (slaughter, retail, and consumption). The rapid increase in the availability of genome sequences and comparative genomic data has increased our understanding of the epidemiology and virulence capacity of this organism. Despite such advances, *Campylobacter* remains a poorly characterized microorganism, and many aspects of *Campylobacter* biology remain unexplained.

The incidence of *Campylobacter* infection in humans can be decreased by reducing the level of bacterial colonization in birds at the farm level and avoiding cross-contamination throughout the food chain. This can be achieved by multiple approaches including regular monitoring of poultry; comparison of the different on-farm and post-harvest practices that affect *Campylobacter* occurrence rates in poultry; potential interventions in poultry transport/slaughter house/farm practices; and advanced understanding of water treatment, feed regimes, and supplements.

The development of effective prevention and intervention strategies is severely hampered by our relatively poor understanding of *Campylobacter* biology, compared with some other foodborne pathogens. Elucidating the molecular mechanisms underlying physiology, metabolism, stress adaptation, infection by and virulence of *Campylobacter*, and its interactions with its hosts will help informed decision-making for the reduction and/or elimination of *Campylobacter* in the food chain. Rapid on-farm monitoring of *Campylobacter* and understanding the genetic diversity of the *C. jejuni* are critical for the development of effective control measures. Identification of novel and potent antigenic proteins of *C. jejuni* will enhance our efforts in effective vaccine development. New approaches for vaccine optimization that will assist in improving the *C. jejuni* vaccine and other vaccines under development are needed. Nanoparticle delivery approach can be used for maximum bioavailability of vaccine in host. This effect can be further potentiated by using adjuvant, pre-, and probiotics as well as through combination of approaches.

References

Adkin, A., Hartnett, E., Jordan, L., Newell, D., & Davidson, H. (2006). Use of systematic review to assist the development of *Campylobacter* control strategies in broilers. *Journal of Applied Microbiology, 100*, 306–315.

Allain, V., Chemaly, M., Laisney, M. J., Rouxel, S., Quesne, S., & Le, B. S. (2014). Prevalence of and risk factors for *Campylobacter* colonization in broiler flocks at the end of the rearing period in France. *British Poultry Science, 55*, 452–459.

Allen, V. M., Ridley, A. M., Harris, J. A., Newell, D. G., & Powell, L. (2011). Influence of production system on the rate of onset of *Campylobacter* colonization in chicken flocks reared extensively in the United Kingdom. *British Poultry Science, 52*, 30–39.

Allen, V. M., Weaver, H., Ridley, A. M., Harris, J. A., Sharma, M., Emery, J., et al. (2008). Sources and spread of thermophilic *Campylobacter* spp. during partial depopulation of broiler chicken flocks. *Journal of Food Protection, 71*, 264–270.

Annamalai, T., Pina-Mimbela, R., Kumar, A., Binjawadagi, B., Liu, Z., Renukaradhya, G. J., et al. (2013). Evaluation of nanoparticle-encapsulated outer membrane proteins for the control of *Campylobacter jejuni* colonization in chickens. *Poultry Science, 92*, 2201–2211.

Ansari-Lari, M., Hosseinzadeh, S., Shekarforoush, S. S., Abdollahi, M., & Berizi, E. (2011). Prevalence and risk factors associated with *Campylobacter* infections in broiler flocks in Shiraz, southern Iran. *International Journal of Food Microbiology, 144*, 475–479.

Arsenault, J., Letellier, A., Quessy, S., Normand, V., & Boulianne, M. (2007). Prevalence and risk factors for *Salmonella* spp. and *Campylobacter* spp. caecal colonization in broiler chicken and turkey flocks slaughtered in Quebec, Canada. *Preventive Veterinary Medicine, 81*, 250–264.

Bahrndorff, S., Rangstrup-Christensen, L., Nordentoft, S., & Hald, B. (2013). Foodborne disease prevention and broiler chickens with reduced *Campylobacter* infection. *Emerging Infectious Diseases, 19*, 425–430.

Barrios, P. R., Reiersen, J., Lowman, R., Bisaillon, J. R., Michel, P., Fridriksdottir, V., et al. (2006). Risk factors for *Campylobacter* spp. colonization in broiler flocks in Iceland. *Preventive Veterinary Medicine, 74*, 264–278.

Behnsen, J., Deriu, E., Sassone-Corsi, M., & Raffatellu, M. (2013). Probiotics: Properties, examples, and specific applications. *Cold Spring Harbor Perspectives in Medicine, 3*, a010074. https://doi.org/10.1101/cshperspect.a010074

Berghaus, R. D., Thayer, S. G., Law, B. F., Mild, R. M., Hofacre, C. L., & Singer, R. S. (2013). Enumeration of *Salmonella* and *Campylobacter* spp. in environmental farm samples and processing plant carcass rinses from commercial broiler chicken flocks. *Applied and Environmental Microbiology, 79*, 4106–4114.

Bouwknegt, M., van de Giessen, A. W., Dam-Deisz, W. D., Havelaar, A. H., Nagelkerke, N. J., & Henken, A. M. (2004). Risk factors for the presence of *Campylobacter* spp. in Dutch broiler flocks. *Preventive Veterinary Medicine, 62*, 35–49.

Bratz, K., Gölz, G., Janczyk, P., Nöckler, K., & Alter, T. (2015). Analysis of in vitro and in vivo effects of probiotics against *Campylobacter* spp. *Berliner und Münchener Tierärztliche Wochenschrift, 128*, 155–162.

Buckley, A. M., Wang, J., Hudson, D. L., Grant, A. J., Jones, M. A., Maskell, D. J., et al. (2010). Evaluation of live-attenuated *Salmonella* vaccines expressing *Campylobacter* antigens for control of *C. jejuni* in poultry. *Vaccine, 28*, 1094–1105.

Buhr, R. J., Cox, N. A., Stern, N. J., Musgrove, M. T., Wilson, J. L., & Hiett, K. L. (2002). Recovery of *Campylobacter* from segments of the reproductive tract of broiler breeder hens. *Avian Diseases, 46*, 919–924.

Bull, S. A., Allen, V. M., Domingue, G., Jorgensen, F., Frost, J. A., Ure, R., et al. (2006). Sources of *Campylobacter* spp. colonizing housed broiler flocks during rearing. *Applied and Environmental Microbiology, 72*, 645–652.

Byrd, J. A., Hargis, B. M., Caldwell, D. J., Bailey, R. H., Herron, K. L., Mac Reynolds, J. L., et al. (2001). Mint: Effect of lactic acid administration in the drinking water during preslaughter feed withdrawal on *Salmonella* and *Campylobacter* contamination of broilers. *Poultry Science, 80*, 278–283.

Callicott, K. A., Fridriksdottir, V., Reiersen, J., Lowman, R., Bisaillon, J. R., Gunnarsson, E., et al. (2006). Lack of evidence for vertical transmission of *Campylobacter* spp. in chickens. *Applied and Environmental Microbiology, 72*, 5794–5798.

Carvalho, C. M., Gannon, B. W., Halfhide, D. E., Santos, S. B., Hayes, C. M., Roe, J. M., et al. (2010). The in vivo efficacy of two administration routes of a phage cocktail to reduce numbers of Campylobacter coli and *Campylobacter jejuni* in chickens. *BMC Microbiology, 10*, 232.

Cawthraw, S. A., & Newell, D. G. (2010). Investigation of the presence and protective effects of maternal antibodies against *Campylobacter jejuni* in chickens. *Avian Diseases, 54*, 86–93.

Chaloner, G., Wigley, P., Humphrey, S., Kemmett, K., Lacharme-Lora, L., Humphrey, T., et al. (2014). Dynamics of dual infection with *Campylobacter jejuni* strains in chickens reveals distinct strain-to-strain variation in infection ecology. *Applied and Environmental Microbiology, 80*, 6366–6372.

Cole, K., Farnell, M. B., Donoghue, A. M., Stern, N. J., Svetoch, E. A., Eruslanov, B. N., et al. (2006). Bacteriocins reduce Campylobacter colonization and alter gut morphology in Turkey poults. *Poultry Science, 85*, 1570–1575.

Connerton, I. F., Connerton, P. L., Barrow, P., Seal, B. S., & Atterbury, R. J. (2008). Bacteriophage therapy and campylobacter. In I. Nachamkin, C. M. Szymanski, & M. J. Blaser (Eds.), *Campylobacter* (3rd ed., pp. 679–693). Washington, DC: American Society for Microbiology.

Cotter, P. D., Hill, C., & Ross, R. P. (2005). Bacteriocins: Developing innate immunity for food. *Nature Reviews. Microbiology, 3*, 777–788.

Cox, N. A., Richardson, L. J., Maurer, J. J., Berrang, M. E., Fedorka-Cray, P. J., Buhr, R. J., et al. (2012). Evidence for horizontal and vertical transmission in Campylobacter passage from hen to her progeny. *Journal of Food Protection, 75*, 1896–1902.

Cox, N. A., Stern, N. J., Wilson, J. L., Musgrove, M. T., Buhr, R. J., & Hiett, K. L. (2002). Isolation of Campylobacter spp. from semen samples of commercial broiler breeder roosters. *Avian Diseases, 46*, 717–720.

de Zoete, M. R., van Putten, J. P., & Wagenaar, J. A. (2007). Vaccination of chickens against Campylobacter. *Vaccine, 25*, 5548–5557.

Ellis-Iversen, J., Ridley, A., Morris, V., Sowa, A., Harris, J., Atterbury, R., et al. (2012). Persistent environmental reservoirs on farms as risk factors for Campylobacter in commercial poultry. *Epidemiology and Infection, 140*, 916–924.

El-Shibiny, A., Scott, A., Timms, A., Metawea, Y., Connerton, P., & Connerton, I. (2009). Application of a group II Campylobacter bacteriophage to reduce strains of *Campylobacter jejuni* and Campylobacter coli colonizing broiler chickens. *Journal of Food Protection, 72*, 733–740.

Elvers, K. T., Morris, V. K., Newell, D. G., & Allen, V. M. (2011). Molecular tracking, through processing, of Campylobacter strains colonizing broiler flocks. *Applied and Environmental Microbiology, 77*, 5722–5729.

Fanelli, A., Agazzi, A., Alborali, G. L., Pilotto, A., Bontempo, V., & Dell'orto, V. (2015). Prevalence reduction of pathogens in poultry fed with Saccharomyces cerevisiae. *Biotechnologie, Agronomie, Société et Environnement, 19*, 3–10.

Fischer, S., Kittler, S., Klein, G., & Glunder, G. (2013). Impact of a single phage and a phage cocktail application in broilers on reduction of *Campylobacter jejuni* and development of resistance. *PLoS ONE, 8*, e78543.

Gibbens, J. C., Pascoe, S. J. S., Evans, S. J., Davies, R. H., & Sayers, A. R. (2001). Mint: A trial of biosecurity as a means to control *Campylobacter* infection of broiler chickens. *Preventive Veterinary Medicine, 48*, 85–99.

Glünder, G., Spiering, N., & Hinz, K. (1997). Investigations on parenteral immunization of chickens with a *Campylobacter* mineral oil vaccine. In B. Nagy & R. Mulder (Eds.), *COST action 97 pathogenic micro-organisms in poultry and eggs* (pp. 247–253). Budapest, Hungary: European Commission.

Golz, G., Rosner, B., Hofreuter, D., Josenhans, C., Kreienbrock, L., Lowenstein, A., et al. (2014). Relevance of *Campylobacter* to public health—the need for a One Health approach. *International Journal of Medical Microbiology, 304*, 817–823.

Gormley, F. J., Bailey, R. A., Watson, K. A., McAdam, J., Avendano, S., Stanley, W. A., et al. (2014). *Campylobacter* colonization and proliferation in the broiler chicken upon natural field challenge is not affected by the bird growth rate or breed. *Applied and Environmental Microbiology, 80*, 6733–6738.

Hald, B., Skovgard, H., Bang, D. D., Pedersen, K., Dybdahl, J., Jespersen, J. B., et al. (2004). Flies and *Campylobacter* infection of broiler flocks. *Emerging Infectious Diseases, 10*, 1490–1492.

Hansson, I., Engvall, E. O., Vagsholm, I., & Nyman, A. (2010). Risk factors associated with the presence of *Campylobacter*-positive broiler flocks in Sweden. *Preventive Veterinary Medicine, 96*, 114–121.

Hansson, I., Vagsholm, I., Svensson, L., & Engvall, E. O. (2007). Correlations between *Campylobacter* spp. prevalence in the environment and broiler flocks. *Journal of Applied Microbiology, 103*, 640–649.

Hechard, Y., & Sahl, H. G. (2002). Mode of action of modified and unmodified bacteriocins from Gram-positive bacteria. *Biochimie, 84*, 545–557.

Helmy, Y. A., Kassem, I. I., Kumar, A., & Rajashekara, G. (2017). In vitro evaluation of the impact of the probiotic E. coli nissle 1917 on *Campylobacter jejuni*'s invasion and intracellular survival in human colonic cells. *Frontiers in Microbiology, 8*, 1588. https://doi.org/10.3389/fmicb.2017.01588

Herman, L., Heyndrickx, M., Grijspeerdt, K., Vandekerchove, D., Rollier, I., & De, Z. L. (2003). Routes for *Campylobacter* contamination of poultry meat: Epidemiological study from hatchery to slaughterhouse. *Epidemiology and Infection, 131*, 1169–1180.

Hermans, D., Pasmans, F., Messens, W., Martel, A., Van, I. F., Rasschaert, G., et al. (2012). Poultry as a host for the zoonotic pathogen *Campylobacter* jejuni. *Vector Borne and Zoonotic Diseases, 12*, 89–98.

Hermans, D., Van, D. K., Messens, W., Martel, A., Van, I. F., Haesebrouck, F., et al. (2011). *Campylobacter* control in poultry by current intervention measures ineffective: Urgent need for intensified fundamental research. *Veterinary Microbiology, 152*, 219–228.

Hiett, K. L., Cox, N. A., Buhr, R. J., & Stern, N. J. (2002). Genotype analyses of *Campylobacter* isolated from distinct segments of the reproductive tracts of broiler breeder hens. *Current Microbiology, 45*, 400–404.

Hilmarsson, H., Thormar, H., Thrainsson, J. H., Gunnarsson, E., & Dadadottir, S. (2006). Effect of glycerol monocaprate (monocaprin) on broiler chickens: An attempt at reducing intestinal Campylobacter infection. *Poultry Science, 85*, 588–592.

Horrocks, S. M., Anderson, R. C., Nielsbet, D. J., & Ricke, S. C. (2009). Incidence and ecology of *Campylobacter jejuni* and *coli* in animals. *Anaerobe, 15*, 18–25.

Huang, J. L., Yin, Y. X., Pan, Z. M., Zhang, G., Zhu, A. P., Liu, X. F., et al. (2010). Intranasal immunization with chitosan/pCAGGS-flaA nanoparticles inhibits *Campylobacter jejuni* in a White Leghorn model. *Journal of Biomedicine & Biotechnology, 2010*, 589476. https://doi.org/10.1155/2010/589476

Humphrey, S., Chaloner, G., Kemmett, K., Davidson, N., Williams, N., Kipar, A., et al. (2014). *Campylobacter jejuni* is not merely a commensal in commercial broiler chickens and affects bird welfare. *MBio, 5*, e01364–e01314.

Jacobs-Reitsma, W., Lyhs, U., & Wagenaar, J. (2008). *Campylobacter* in the food supply. In I. Nachamkin, C. M. Szymanski, & M. Blaser (Eds.), *Campylobacter* (3rd ed., pp. 627–644). Washington, DC: ASM Press.

Janez, N., & Loc-Carrillo, C. (2013). Use of phages to control *Campylobacter* spp. *Journal of Microbiological Methods, 95*, 68–75.

Jones, C. (2005). Vaccines based on the cell surface carbohydrates of pathogenic bacteria. *Anais da Academia Brasileira de Ciências, 77*, 293–324.

Jonsson, M. E., Chriel, M., Norstrom, M., & Hofshagen, M. (2012). Effect of climate and farm environment on *Campylobacter* spp. colonisation in Norwegian broiler flocks. *Preventive Veterinary Medicine, 107*, 95–104.

Jore, S., Viljugrein, H., Brun, E., Heier, B. T., Borck, B., Ethelberg, S., et al. (2010). Trends in *Campylobacter* incidence in broilers and humans in six European countries, 1997–2007. *Preventive Veterinary Medicine, 93*, 33–41.

Kalupahana, R. S., Kottawatta, K. S., Kanankege, K. S., Van Bergen, M. A., Abeynayake, P., & Wagenaar, J. A. (2013). Colonization of *Campylobacter* spp. in broiler chickens and laying hens reared in tropical climates with low biosecurity housing. *Applied and Environmental Microbiology, 79*, 393–395.

Kashoma, I. P. B., Kumar, A., Sanad, Y. M., Gebreyes, W., Kazwala, R. R., Garabed, R., et al. (2014). Phenotypic and genotypic diversity of thermophilic *Campylobacter* spp. in commercial Turkey flocks: A longitudinal study. *Foodborne Pathogens and Disease, 11*, 850–860.

Kassem, I. I., Sanad, Y., Gangaiah, D., Lilburn, M., LeJeune, J., & Rajashekara, G. (2010). Use of bioluminescence imaging to monitor *Campylobacter* survival in chicken litter. *Journal of Applied Microbiology, 109*, 1988–1997.

Kemmett, K. (2015). Probiotics and enzymes: A good combination: Feed science. *AFMA Matrix, 24*, 35–37.

Khoury, C. A., & Meinersmann, R. J. (1995). A genetic hybrid of the *Campylobacter jejuni* flaA gene with LT-B of Escherichia coli and assessment of the efficacy of the hybrid protein as an oral chicken vaccine. *Avian Diseases, 39*, 812–820.

Kittler, S., Fischer, S., Abdulmawjood, A., Glunder, G., & Klein, G. (2013). Effect of bacteriophage application on *Campylobacter jejuni* loads in commercial broiler flocks. *Applied and Environmental Microbiology, 79*, 7525–7533.

Knudsen, K. N., Bang, D. D., Andresen, L. O., & Madsen, M. (2006). *Campylobacter jejuni* strains of human and chicken origin are invasive in chickens after oral challenge. *Avian Diseases, 50*, 10–14.

Kobierecka, P. A., Olech, B., Książek, M., Derlatka, K., Adamska, I., Majewski, P. M., et al. (2016). Cell wall anchoring of the *Campylobacter* antigens to *Lactococcus lactis*. *Frontiers in Microbiology*. https://doi.org/10.3389/fmicb.2016.00165

Lam, K. M., DaMassa, A. J., Morishita, T. Y., Shivaprasad, H. L., & Bickford, A. A. (1992). Pathogenicity of *Campylobacter jejuni* for Turkeys and chickens. *Avian Diseases, 36*, 359–363.

Laniewski, P., Kuczkowski, M., Chrzastek, K., Wozniak, A., Wyszynska, A., Wieliczko, A., et al. (2014). Evaluation of the immunogenicity of *Campylobacter jejuni* CjaA protein delivered by Salmonella enterica sv. Typhimurium strain with regulated delayed attenuation in chickens. *World Journal of Microbiology and Biotechnology, 30*, 281–292.

Layton, S. L., Morgan, M. J., Cole, K., Kwon, Y. M., Donoghue, D. J., Hargis, B. M., et al. (2011). Evaluation of Salmonella-vectored Campylobacter peptide epitopes for reduction of *Campylobacter jejuni* in broiler chickens. *Clinical and Vaccine Immunology, 18*, 449–454.

Lin, J. (2009). Novel approaches for campylobacter control in poultry: Review. *Foodborne Pathogens and Disease, 6*(7), 755–765.

Line, J. E. (2002). *Campylobacter* and *Salmonella* populations associated with chickens raised on acidified litter. *Poultry Science, 81*, 1473–1477.

Line, J. E., Svetoch, E. A., Eruslanov, B. V., Perelygin, V. V., Mitsevich, E. V., Mitsevich, I. P., et al. (2008). Isolation and purification of enterocin E-760 with broad antimicrobial activity against gram-positive and gram-negative bacteria. *Antimicrobial Agents and Chemotherapy, 52*, 1094–1100.

Loc Carrillo, C., Atterbury, R. J., el-Shibiny, A., Connerton, P. L., Dillon, E., Scott, A., et al. (2005). Bacteriophage therapy to reduce *Campylobacter jejuni* colonization of broiler chickens. *Applied and Environmental Microbiology, 71*, 6554–6563.

Luangtongkum, T., Morishita, T. Y., Ison, A. J., Huang, S., McDermott, P. F., & Zhang, Q. (2006). Effect of conventional and organic production practices on the prevalence and antimicrobial resistance of *Campylobacter* spp. in poultry. *Applied and Environmental Microbiology, 72*, 3600–3607.

Lyngstad, T. M., Jonsson, M. E., Hofshagen, M., & Heier, B. T. (2008). Mint: Risk factors associated with the presence of *Campylobacter* species in Norwegian broiler flocks. *Poultry Science, 87*, 1987–1994.

Messaoudi, S., Kergourlay, G., Dalgalarrondo, M., Choiset, Y., Ferchichi, M., Prevost, H., et al. (2012). Purification and characterization of a new bacteriocin active against *Campylobacter* produced by *Lactobacillus salivarius* SMXD51. *Food Microbiology, 32*, 129–134.

Meunier, M., Guyard-Nicodeme, M., Dory, D., & Chemaly, M. (2015). Control strategies against *Campylobacter* at the poultry production level: Biosecurity measures, feed additives and vaccination. *Journal of Applied Microbiology, 120*, 1139–1173.

Mohan, V. (2015). The role of probiotics in the inhibition of *Campylobacter jejuni* colonization and virulence attenuation. *European Journal of Clinical Microbiology & Infectious Diseases, 34*, 1503–1513. https://doi.org/10.1007/s10096-015-2392-z

Mohyla, P., Bilgili, S. F., Oyarzabal, O. A., Warf, C. C., & Kemp, G. K. (2007). Mint application of acidified sodium chlorite in the drinking water to control *Salmonella* serotype *Typhimurium* and *Campylobacter jejuni* in commercial broilers. *Journal of Applied Poultry Research, 16*, 45–51.

Muller, A., Thomas, G. H., Horler, R., Brannigan, J. A., Blagova, E., Levdikov, V. M., et al. (2005). An ATP-binding cassette-type cysteine transporter in *Campylobacter jejuni* inferred from the structure of an extracytoplasmic solute receptor protein. *Molecular Microbiology, 57*, 143–155.

Nather, G., Alter, T., Martin, A., & Ellerbroek, L. (2009). Analysis of risk factors for *Campylobacter* species infection in broiler flocks. *Poultry Science, 88*, 1299–1305.

Neal-McKinney, J. M., Samuelson, D. R., Eucker, T. P., Nissen, M. S., Crespo, R., & Konkel, M. E. (2014). Reducing *Campylobacter jejuni* colonization of poultry via vaccination. *PLoS ONE, 9*, e114254.

Newell, D. G., & Wagenaar, J. A. (2000). Poultry infections and their control at the farm level. In I. Nachamkin & M. J. Blaser (Eds.), *Campylobacter* (2nd ed., pp. 497–509). Washington, DC: ASM Press.

Noor, S. M., Husband, A. J., & Widders, P. R. (1995). *In vivo* oral vaccination with *Campylobacter jejuni* establishes early development of intestinal immunity in chickens. *British Poultry Science, 36*, 563–573.

Nothaft, H., Davis, B., Lock, Y. Y., Perez-Munoz, M. E., Vinogradov, E., Walter, J., et al. (2016). Engineering the Campylobacter jejuni N-glycan to create an effective chicken vaccine. *Scientific Reports, 6*, 26511.

Nothaft, H., Perez-Muño, M. E., Gouveia, G. J., Duar, R. M., Wanford, J. J., Lango-Scholey, L., et al. (2017) Co-administration of the *Campylobacter jejuni* N-glycan based vaccine with probiotics improves vaccine performance in broiler chickens. *Applied and Environmental Microbiology*. https://doi.org/10.1128/AEM.01523-17

Nothaft, H., Scott, N. E., Vinogradov, E., Liu, X., Hu, R., Beadle, B., et al. (2012). Diversity in the protein N-glycosylation pathways among *Campylobacter* species. *Molecular & Cellular Proteomics, 11*, 1203–1219.

Patriarchi, A., Fox, A., Maunsell, B., Fanning, S., & Bolton, D. (2011). Molecular characterization and environmental mapping of *Campylobacter* isolates in a subset of intensive poultry flocks in Ireland. *Foodborne Pathogens and Disease, 8*, 99–108.

Rice, B. E., Rollins, D. M., Mallinson, E. T., Carr, L., & Joseph, S. W. (1997). *Campylobacter jejuni* in broiler chickens: Colonization and humoral immunity following oral vaccination and experimental infection. *Vaccine, 15*, 1922–1932.

Ridley, A., Morris, V., Gittins, J., Cawthraw, S., Harris, J., Edge, S., et al. (2011). Potential sources of Campylobacter infection on chicken farms: Contamination and control of broiler-harvesting equipment, vehicles and personnel. *Journal of Applied Microbiology, 111*, 233–244.

Riley, M. A., & Wertz, J. E. (2002). Bacteriocins: Evolution, ecology, and application. *Annual Review of Microbiology, 56*, 117–137.

Ring, M., Zychowska, M. A., & Stephan, R. (2005). Dynamics of *Campylobacter* spp. spread investigated in 14 broiler flocks in Switzerland. *Avian Diseases, 49*, 390–396.

Rivoal, K., Ragimbeau, C., Salvat, G., Colin, P., & Ermel, G. (2005). Genomic diversity of *Campylobacter coli* and *Campylobacter jejuni* isolates recovered from free-range broiler farms and comparison with isolates of various origins. *Applied and Environmental Microbiology, 71*, 6216–6227.

Sahin, O., Fitzgerald, C., Stroika, S., Zhao, S., Sippy, R. J., Kwan, P., et al. (2012). Molecular evidence for zoonotic transmission of an emergent, highly pathogenic Campylobacter jejuni clone in the United States. *Journal of Clinical Microbiology, 50*, 680–687.

Sahin, O., Kassem, I. I., Shen, Z., Lin, J., Rajashekara, G., & Zhang, Q. (2015). *Campylobacter* in poultry: Ecology and potential interventions. *Avian Diseases, 59*(2), 185–200.

Sahin, O., Luo, N., Huang, S., & Zhang, Q. (2003). Effect of Campylobacter-specific maternal antibodies on *Campylobacter jejuni* colonization in young chickens. *Applied and Environmental Microbiology, 69*, 5372–5379.

Sahin, O., Morishita, T. Y., & Zhang, Q. (2002). *Campylobacter* colonization in poultry: Sources of infection and modes of transmission. *Animal Health Research Reviews, 3*, 95–105.

Saint-Cyr, M. J., Guyard-Nicodème, M., Messaoudi, S., Chemaly, M., Cappelier, J. M., & Dousset, X. (2016). Recent advances in screening of anti-*Campylobacter* activity in probiotics for use in poultry. *Frontiers in Microbiology, 7*, 553. https://doi.org/10.3389/fmicb.2016.00553

Shane, S. M., Gifford, D. H., & Yogasundram, K. (1986). *Campylobacter jejuni* contamination of eggs. *Veterinary Research Communications, 10*, 487–492.

Shanker, S., Lee, A., & Sorrell, T. C. (1986). *Campylobacter jejuni* in broilers: The role of vertical transmission. *The Journal of Hygiene, 96*, 153–159.

Sherman, P. M., Johnson-Henry, K. C., Yeung, H. P., Ngo, P. S., Goulet, J., & Tompkins, T. A. (2005). Probiotics reduce enterohemorrhagic *Escherichia coli* O157:H7- and enteropathogenic *E. coli* O127:H6-induced changes in polarized T84 epithelial cell monolayers by reducing bacterial adhesion and cytoskeletal rearrangements. *Infection and Immunity, 73*, 5183–5188. https://doi.org/10.1128/IAI.73.8.5183-5188.2005

Silva, J., Leite, D., Fernandes, M., Mena, C., Gibbs, P. A., & Teixeira, P. (2011). *Campylobacter* spp. as a foodborne pathogen: A review. *Frontiers in Microbiology, 2*, 200. https://doi.org/10.3389/fmicb.2011.00200

Sit, C. S., & Vederas, J. C. (2008). Approaches to the discovery of new antibacterial agents based on bacteriocins. *Biochemistry and Cell Biology, 86*, 116–123.

Sommer, H. M., Heuer, O. E., Sorensen, A. I., & Madsen, M. (2013). Analysis of factors important for the occurrence of *Campylobacter* in Danish broiler flocks. *Preventive Veterinary Medicine, 111*, 100–111.

Sparks, N. H. C. (2009). Mint: The role of the water supply system in the infection and control of *Campylobacter* in chicken. *World Poultry Science Journal, 65*, 459–474.

Stef, L. (2016). Evaluation of the administration effects of probiotics against *Campylobacter jejuni* on the immune system of broiler chickens. *Animal Science & Biotechnologies, 49*, 16–21. https://doi.org/10.1371/journal.ppat.1004264

Stern, N. J., Cox, N. A., & Musgrove, M. T. (2001). Incidence and levels of *Campylobacter* in broilers after exposure to an inoculated seeder bird. *Journal of Applied Poultry Research, 10*, 315–318.

Stern, N. J., & Pretanik, J. (2006). Counts of *Campylobacter* spp. on U.S. broiler carcasses. *Journal of Food Protection, 69*, 1034–1039.

Stern, N. J., Robach, M. C., Cox, N. A., & Musgrove, M. T. (2002). Effect of drinking water chlorination on *Campylobacter* spp. colonization of broilers. *Avian Diseases, 46*, 401–404.

Stern, N. J., Svetoch, E. A., Eruslanov, B. V., Kovalev, Y. N., Volodina, L. I., Perelygin, V. V., et al. (2005). Paenibacillus polymyxa purified bacteriocin to control *Campylobacter jejuni* in chickens. *Journal of Food Protection, 68*, 1450–1453.

Stern, N. J., Svetoch, E. A., Eruslanov, B. V., Perelygin, V. V., Mitsevich, E. V., Mitsevich, I. P., et al. (2006). Isolation of a *Lactobacillus salivarius* strain and purification of its bacteriocin, which is inhibitory to *Campylobacter jejuni* in the chicken gastrointestinal system. *Antimicrobial Agents and Chemotherapy, 50*, 3111–3116.

Svetoch, E. A., Eruslanov, B. V., Levchuk, V. P., Perelygin, V. V., Mitsevich, E. V., Mitsevich, I. P., et al. (2011). Isolation of *Lactobacillus salivarius* 1077 (NRRL B-50053) and characterization of its bacteriocin, including the antimicrobial activity spectrum. *Applied and Environmental Microbiology, 77*, 2749–2754.

Svetoch, E. A., Eruslanov, B. V., Perelygin, V. V., Mitsevich, E. V., Mitsevich, I. P., Borzenkov, V. N., et al. (2008). Diverse antimicrobial killing by *Enterococcus faecium* E 50-52 bacteriocin. *Journal of Agricultural and Food Chemistry, 56*, 1942–1948.

Svetoch, E. A., Stern, N. J., Eruslanov, B. V., Kovalev, Y. N., Volodina, L. I., Perelygin, V. V., et al. (2005). Isolation of *Bacillus circulans* and *Paenibacillus polymyxa* strains inhibitory to *Campylobacter jejuni* and characterization of associated bacteriocins. *Journal of Food Protection, 68*, 11–17.

Szymanski, C. M., St Michael, F., Jarrell, H. C., Li, J. J., Gilbert, M., Larocque, S., et al. (2003). Detection of conserved N-linked glycans and phase-variable lipooligosaccharides and capsules from campylobacter cells by mass spectrometry and high resolution magic angle spinning NMR spectroscopy. *The Journal of Biological Chemistry, 278*, 24509–24520.

Theoret, J. R., Coope, K. K., Zekarias, B., Roland, K. L., Law, B. F., Curtiss, R., et al. (2012). The *Campylobacter jejuni* Dps homologue is important for in vitro biofilm formation and cecal colonization of poultry and may serve as a protective antigen for vaccination. *Clinical and Vaccine Immunology, 19*, 1426–1431.

Thomrongsuwannakij, T., Chuanchuen, R., & Chansiripornchai, N. (2016). Identification of competitive exclusion and its ability to protect against *Campylobacter jejuni* in broilers. *The Thai Journal of Veterinary Medicine, 46*, 279–286.

Umaraw, P., Prajapati, A., Verma, A. K., Pathak, V., & Singh, V. P. (2015). Control of *Campylobacter* in poultry industry from farm to poultry processing unit-a review. *Critical Reviews in Food Science and Nutrition*. https://doi.org/10.1080/10408398.2014.935847

van de Giessen, A., Mazurier, S. I., Jacobs-Reitsma, W., Jansen, W., Berkers, P., Ritmeester, W., et al. (1992). Study on the epidemiology and control of *Campylobacter jejuni* in poultry broiler flocks. *Applied and Environmental Microbiology, 58*, 1913–1917.

van de Giessen, A. W., Tilburg, J. J., Ritmeester, W. S., & Van der, P. J. (1998). Reduction of *Campylobacter* infections in broiler flocks by application of hygiene measures. *Epidemiology and Infection, 121*, 57–66.

van Gerwe, T., Bouma, A., Klinkenberg, D., Wagenaar, J. A., Jacobs-Reitsma, W. F., & Stegeman, A. (2010). Medium chain fatty acid feed supplementation reduces the probability of *Campylobacter jejuni* colonization in broilers. *Veterinary Microbiology, 143*, 314–318.

Van Overbeke, I., Duchateau, L., De Zutter, L., Albers, G., & Ducatelle, R. (2006). A comparison survey of organic and conventional broiler chickens for infectious agents affecting health and food safety. *Avian Diseases, 50*, 196–200.

Wagenaar, J. A., Jacobs-Reitsma, W., Hofshagen, M., & Newell, D. G. (2008). Poultry colonization with *Campylobacter* and its control at the primary production level. In I. Nachamkin, C. M. Szymanski, & M. J. Blaser (Eds.), *Campylobacter* (3rd ed., pp. 667–678). Washington, DC: American Society for Microbiology.

Wagenaar, J. A., Van Bergen, M. A., Mueller, M. A., Wassenaar, T. M., & Carlton, R. M. (2005). Phage therapy reduces *Campylobacter jejuni* colonization in broilers. *Veterinary Microbiology, 109*, 275–283.

Widders, P. R., Thomas, L. M., Long, K. A., Tokhi, M. A., Panaccio, M., & Apos, E. (1998). The specificity of antibody in chickens immunised to reduce intestinal colonisation with *Campylobacter jejuni*. *Veterinary Microbiology, 64*, 39–50.

Wittwer, M., Keller, J., Wassenaar, T. M., Stephan, R., Howald, D., Regula, G., et al. (2005). Genetic diversity and antibiotic resistance patterns in a Campylobacter population isolated from poultry farms in Switzerland. *Applied and Environmental Microbiology, 71*, 2840–2847.

World Health Organization (WHO). (2001). Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria: Report of a Joint FAO WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria.

World Health Organization (WHO). (2015). *WHO estimates of the global burden of foodborne diseases*. Geneva: WHO.

Wyszynska, A., Raczko, A., Lis, M., & Jagusztyn-Krynicka, E. K. (2004). Oral immunization of chickens with avirulent *Salmonella* vaccine strain carrying *C. jejuni* 72Dz/92 cjaA gene elicits specific humoral immune response associated with protection against challenge with wild-type Campylobacter. *Vaccine, 22*, 1379–1389.

Zhang, Q., & Sahin, O. (2013). Campylobacteriosis. In D. E. Swayne, J. R. Glisson, L. R. McDougald, L. K. Nolan, D. L. Suarez, & V. L. Nair (Eds.), *Diseases of poultry* (13th ed., pp. 737–750). Ames, IA: Wiley.

Zimmer, M., Barnhart, H., Idris, U., & Lee, M. D. (2003). Detection of *Campylobacter jejuni* strains in the water lines of a commercial broiler house and their relationship to the strains that colonized the chickens. *Avian Diseases, 47*, 101–107.

Ziprin, R. L., Hume, M. E., Young, C. R., & Harvey, R. B. (2002). Inoculation of chicks with viable non-colonizing strains of *Campylobacter jejuni*: Evaluation of protection against a colonizing strain. *Current Microbiology, 44*, 221–223.

Chapter 10
Applications of "Omics" Technologies to Study Gut Health in Poultry

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10.1 Introduction

Over the past 50 years, the United States poultry industry has transformed itself from a backyard business into one of the most advanced sectors of agriculture, supplying products to customers globally. At present, the United States is the largest poultry producer and second largest exporter of poultry meat in the world (USDA-ERS 2012), with its total value exceeding $20 billion, primarily from broiler production, followed by eggs, turkey, and other poultry products (USDA-ERS 2012). Despite these improvements, the microbiological safety of poultry products remains a challenge for the industry. Between 1998 and 2008, contaminated poultry products were responsible for the majority (18.9% of total) of foodborne outbreaks (MMWR, Centers for Disease Control and Prevention 2013). Since 2008, the consumption of contaminated poultry products accounted for at least 14 major foodborne outbreaks resulting in illnesses to over a million people (Centers for Disease Control and Prevention 2014; Scallan et al. 2011). *Salmonella* and *Campylobacter* are two common foodborne pathogens that are responsible for the majority of these illnesses. Chickens act as the reservoir host for these pathogens (Bakshi et al. 2003; Anonymous 2005), wherein the bacteria colonize the chicken gut (especially the ceca), thereby leading to contamination of carcass during slaughter and subsequent

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human infections (Keller et al. 1995; Cagri et al. 2004). The most commonly detected *Salmonella* serovars in chicken that are associated with human infections include *Salmonella enterica* serovar Enteritidis, *Salmonella* Typhimurium, and *Salmonella* Heidelberg (Foley et al. 2011). In the case of *Campylobacter* spp., humans are most frequently sickened by *Campylobacter jejuni* (~90%), followed by *Campylobacter coli* (~10%) (Gillespie et al. 2003; Taboada et al. 2013). The healthcare cost associated with poultry-related foodborne pathogens is greater than $5 billion each year (USDA-ERS 2012). Therefore, it is important to develop strategies for reducing pathogen colonization in chickens as a first step toward food safety.

The poultry industry has implemented various genetic selection strategies, management approaches, and dietary modifications with an aim to improve performance or disease resistance in chickens (Emmerson 1997; Lumpkins et al. 2010). With recent advancements in next-generation sequencing, the chicken gut has become the focus of extensive research both for improving productivity (better feed utilization, feed conversion ratio) and developing resistance against enteric diseases caused by pathogenic microbes (Park et al. 2016; Roto et al. 2015; Waite and Taylor 2015). Gut health encompasses a plethora of interrelated factors such as nutrient digestion, absorption, epithelial barrier function, gut microbiome, and mucosal immune responses (Kogut and Arsenault 2016). A comprehensive understanding of how these factors interact to bring about overall health and productivity in chickens is still elusive; however, significant scientific endeavors are currently underway to delineate the biochemical cross talk and critical pathways responsible for maintaining gut homeostasis and function. Several research groups have attempted to characterize the gut microbiome of chickens in health and disease (Brisbin et al. 2008; Stanley et al. 2014), and the major bacterial groups that constitute the chicken microbiome along with their potential role in gut metabolism have been identified (Qu et al. 2008; Wei et al. 2013; Oakley et al. 2014). Moreover, the role of microbiota-derived metabolites that signal the host's immune and endocrine system potentially altering host physiology is also under extensive scientific investigations (Belkaid and Hand 2014; Schroeder and Bäckhed 2016).

This book chapter summarizes the current research employing "omics" approaches to study gut health in chickens. In addition, the effect of various feed additives on modulating microbiome/metabolome parameters as they relate to pathogen colonization in chickens is discussed.

10.2 Intestinal Microbiota of Poultry

The gut microbiota represents a stable and specific association between a host and microorganisms, which has developed through a long series of selection, competition, and coevolution (Angelakis et al. 2012; Ley et al. 2008; Yeoman et al. 2011). The spatial heterogeneity and distribution of bacterial communities in the gut are governed by several factors such as microenvironment (pH, redox potential, oxygen levels), nutrient selection, and immune activation (Donaldson et al. 2016; Belkaid and Hand 2014). The host benefits from receiving nutrients catabolized by resident

microbiota from otherwise poorly utilized dietary substrates (Sekirov et al. 2010), whereas the microbiota gains from the availability of an ecosystem that provides a niche and facilitates their survival. In the case of food animals and poultry, gut health is critical for nutrient assimilation, maintenance, growth, and productivity.

As compared to other food animal species (e.g., cattle), the gastrointestinal tract of poultry is an anatomically simple yet physiologically efficient system that helps in feed digestion and nutrient assimilation. Unlike mammalian hosts, the digesta pass through the poultry gut faster with an average transit time of less than 3.5 h (Hughes 2008). This fast passage rate selects for a microbiota with high affinity for mucosal binding and colonization (Pan and Yu 2014; Crhanova et al. 2011). However, the ceca (intestinal out pocketing at the junction of small intestine and colon) have a slow passage rate (~12–20 h) potentially facilitating a longer interaction time for digestion/nutrient assimilation. Several critical physiological functions have been attributed to the ceca including nitrogen cycling, water absorption, carbohydrate fermentation, and production of short-chain fatty acids (SCFA) (Mead 1989; Józefiak et al. 2004; van der Wielen et al. 2000). These anatomic features and physiological demands drive the development of a complex microbiome in the ceca. In fact, most densely populated (10^9 to 10^{11} bacteria/g) microbial community within the chicken gut is found in the ceca (Clench and Mathias 1995; Gong et al. 2006), with more than 2200 operational taxonomic units (Danzeisen et al. 2011) and 3500 genotypes (Qu et al. 2008). Microbial colonization of the gastrointestinal tract in poultry begins immediately post-hatch and establishes by 2 weeks in the small intestine (Amit-Romach et al. 2004; Lu et al. 2003). Thereafter, the microbiota modulates and alters itself based on environmental factors, age, and dietary patterns of the birds (Stanley et al. 2013). The mature chicken gut microbiota consists primarily of bacteria (Wei et al. 2013), followed by archaea (Saengkerdsub et al. 2007a, b) and viruses (Qu et al. 2008). The microbial community is highly diverse with over 1000 bacterial species (Chambers and Gong 2011). Through phylogenetic profiling and 16S rRNA-based sequencing of the intestinal microbiome of poultry, a global census was developed (Wei et al. 2013), which serves as the working framework for describing bacterial diversity in the poultry gut. In total, 13 bacterial phyla were found, including *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* constituting greater than 90% of the intestinal bacteria, with *Firmicutes* being the predominant phyla in the small intestine and cecum of chickens (Rehman et al. 2007; Qu et al. 2008; Danzeisen et al. 2011). The most predominant genera found in poultry (both chickens and turkey) are *Clostridium*, *Ruminococcus*, *Lactobacillus*, and *Bacteroides* with *Lactobacillus* spp. dominating the crop and small intestinal niches (Lu et al. 2003; Apajalahti and Kettunen 2006; Abbas Hilmi et al. 2007). The ceca of poultry consist strictly of anaerobic bacteria (many of unknown bacterial genus) primarily dominated by order *Clostridiales* and families *Lachnospiraceae* and *Ruminococcaceae*. A significant portion of the cecal bacteria also belong to the families *Bifidobacteriaceae* and *Coriobacteriaceceae* (Lu et al. 2003; Bjerrum et al. 2006). These groups of bacteria are known for their ability to utilize dietary polysaccharides (especially components unavailable to the host) for producing SCFA, thereby expanding the overall energy capture from the feed. A positive correlation was reported between cecal *Lachnospiraceae* spp. and feed conversion efficiency in

commercial broilers (Torok et al. 2008; Rinttilä and Apajalahti 2013). However, despite these commonalities in poultry, chickens and turkeys share only 16% similarity at species-equivalent level (Pan and Yu 2014).

10.3 Modulation of Chicken Intestinal Microbiota in Response to Nutritional, Physiological, or Microbial Cues

Extensive microbiota-host cross talk occurs in the poultry gut through nutritional, physiological, and immunological signaling. Scientists are in the beginning stage in deciphering these molecular communications, their effect on the gut microbiome, and their relevance for poultry health and productivity. Gene-based metagenomic studies provide a measure of such metabolic capabilities of the microbiome. Recent findings in this area are presented in this section.

10.3.1 Nutritional/Physiological Signaling

The different sections of poultry gut are inhabited by specialist microbiota adapted to available nutrients and physicochemical conditions of the niche. Most easily digested dietary carbohydrates are absorbed in the proximal gut by the host and the indigestible/residual carbohydrates, and dietary fibers are metabolized by the distal gut bacteria (especially from the ceca) by production of polysaccharide-specific enzymes (Hooper et al. 2002; Rehman et al. 2007). Chickens lack the genes for glycoside hydrolase, polysaccharide lyase, and carbohydrate esterase (Morris 2003) required for carbohydrate metabolism in the gut. Metagenomic studies have shown that genes coding for aforementioned enzymes and various other proteins involved in carbohydrate metabolism are abundantly found (~20% of the gene pool) in the cecal microbiome (Qu et al. 2008; Danzeisen et al. 2011). Breakdown of dietary polysaccharides leads to the production of SCFA in the gut, primarily acetate, followed by propionate and butyrate (Topping and Clifton 2001; Dunkley et al. 2007). The ratio and amount of SCFAs produced depend on the microbial composition and fiber component in poultry diet (Topping and Clifton 2001). The SCFAs are utilized as energy source by the host epithelium and contribute to the development of villus morphology (Panda et al. 2009; Donohoe et al. 2011). They also represent a major source of carbon to the host facilitated by the microbiome (Koutsos and Arias 2006; Tellez et al. 2006). The gut microbiome also contributes to nitrogen metabolism. Genes involved in the metabolism of proteins (9–10% of gene pool), amino acids (8–9%), and nitrogen (1%) have been identified (Qu et al. 2008; Danzeisen et al. 2011). Many of the microbes (e.g., *Lactobacilli*) with fastidious nutritional requirements are usually found in the proximal part of the small intestine, where availability of amino acids, vitamins, and carbohydrates is abundant. These microbes compete

with the host for available nutrients. This leads to the induction of compensatory host mechanisms (secretion of acids, bile salts, antibodies) to balance the growth of microbiota. In the distal part of small intestine, these microbes face strong competition from microbes with lower requirements for easily digested nutrients (e.g., *E. coli*, which does not depend on external amino acids). In this part of the intestine, the microbial metabolism of dietary proteins provides amino acids for host's growth and production (Latshaw and Zhao 2011). However, a high level of bypass protein to the ceca could lead to an increase in protein-fermenting bacteria that negatively affect poultry health by the production of putrefactive protein by-products such as branched chain fatty acids, 3-methyl-indole (skatole), etc. (Smith and Macfarlane 1998).

Several predisposing factors could lead to high protein bypass in poultry. Apajalahti and Bedford (1999) showed that an *Eimeria maxima* challenge to poultry caused an elevation in total biogenic amine levels in the cecum potentially by reducing the integrity and absorptive capacity of the small intestinal epithelium. Similar effects have been observed when birds were administered with feed, high in dietary proteins (Shojadoost et al. 2012). Metabolic processing of proteins to ammonia or urea is another nutritionally inefficient process that leads to their losses in excretion. This also exerts negative effects on the health and productivity of poultry along with environmental and public health concerns (Xin et al. 2011). Several nutritional strategies (e.g., addition of proteolytic enzymes) have been developed to counter these losses (Bregendahl and Roberts 2006); however, microbiome-based strategies to reduce ammonia-associated losses are yet to be explored.

Gene repertoire associated with fatty acid and lipid metabolism has also been identified (Qu et al. 2008). Ding et al. (2016) sequenced the whole gut microbial genomes of two chicken lines (fat and lean) that had undergone long-term divergent selection for abdominal fat pad weight. Results revealed that proportions of *Fusobacteria* (8 vs 18%) and *Proteobacteria* (33 vs 24%) differed significantly between the two lines. Microbial genome functional analysis showed that the gut microbiota was involved in lipid and glycan formation pathways. Citrate cycle and peroxisome proliferator-activated receptor (PPAR) signaling pathways that play crucial roles in lipid storage and metabolism were found to be more prevalent in the fat line than in the lean line of poultry. In another study, Hou et al. (2016) analyzed the gut microbiome of divergently selected lean and fat broiler chicken lines. A significant difference was observed between the lean and fat chicken fecal microbiota structure. Significantly more *Bacteroidetes* was observed in lean broilers. At the genus level, butyrate-producing bacteria (*Subdoligranulum*, *Butyricicoccus*, *Eubacterium*), propionate-producing bacteria (*Bacteroides*), and acetate-producing bacteria (*Blautia*) were reduced in fat line broiler chickens. Since these SCFAs improve barrier function and reduce low-grade inflammation, a precursor for obesity (Costa et al. 2017) and a reduction in these microbiota/compounds could predispose poultry to increased pathogen load and fat accumulation. Follow-up fecal functional metagenomic analysis (KEGG module level) showed that two methanogenesis modules (M00357 and M00567) and pyridoxal biosynthesis (M00124) module were enriched in the fat line broiler chickens which may contribute to fat accumulation. The experimental design of aforementioned studies allows

only correlation-based analysis between modified host physiology and shifts in gut microbiota and does not confirm whether it is the microbiota or the physiology that drives such a selection. However, once a microbiota is selected for a certain physiological phenotype, it could modulate the health/physiology when transplanted into a recipient host (Ridaura et al. 2013; Ley et al. 2005).

10.3.2 Antibiotic Growth Promoters (AGPs)

The food industry has engaged for decades in developing nutritional strategies for improving weight gain in food animals. One such strategy is the use of low-dose antibiotics as feed additives (Frost and Woolcock 1991). Although the precise mechanisms behind the growth-promoting effects of AGPs are unknown, recent evidence suggests that the interactions of AGPs with gut microbiota is a major contributor (Chapman and Johnson 2002; Dibner and Richards 2005; Lin 2011). Dumonceaux et al. (2006) studied changes in the gut microbiota in response to virginiamycin supplementation in broiler chickens using chaperonin 60 (*cpn60*) gene as the target sequence. Virginiamycin increased the abundance of many bacterial targets in the proximal gastrointestinal tract, including lactobacilli (*Lactobacillus crispatus*, *Lactobacillus johnsonii*, *Lactobacillus aviarius*), *Clostridium nexile*, and *Enterococcus cecorum*. In a recent study, Costa et al. (2017) investigated the impact of zinc bacitracin, enramycin, halquinol, virginiamycin, and avilamycin on the cecal microbiota of broiler chickens. Several bacterial genera were identified as representative of usage of each drug. Treatment with enramycin decreased richness and relative abundance of unclassified *Firmicutes*, *Clostridium* XI, and unclassified *Peptostreptococcaceae*, whereas increased abundance of *Clostridium* XIVb and *Anaerosporobacter* spp. occurred. Similar results were observed by Torok et al. (2008) when broiler diets were supplemented with avilamycin, zinc bacitracin, and flavophospholipol. The antimicrobial treatments modulated the composition of gut microbiota. Groups such as *Lachnospiraceae*, *Lactobacillus johnsonii*, *Ruminococcaceae*, *Clostridiales*, and *Oxalobacteraceae* were less prevalent in the guts of chicks fed with antimicrobial supplemented diets. Overall, these studies suggest that AGPs modulate the diversity and structure of microbial population in the poultry gut ultimately resulting in an optimal microbiota that potentially facilitates in more efficient energy harvestation and better growth performance.

10.3.3 Host Immune Interaction with Microbiota

As discussed in the previous section, the association between gut microbiota and host has developed through a long series of selection, competition, and coevolution. Through these bidirectional interactions, the immune system has learnt to respond appropriately to commensal microbiota or pathogens. In turn the microbiota

participates in educating the immune system to function properly (Chow and Mazmanian 2010), and perturbations in early microbiota could affect intestinal immune development (Schokker et al. 2015). Extensive studies in germ-free (GF) mice, in the past decades, have demonstrated the critical role played by gut microbiota in shaping the host intestine immune system (Macpherson and Harris 2004; Grover and Kashyap 2014). Colonization of the poultry gut with microorganisms begins immediately after hatch, eventually leading to the establishment of a complex microbiota (Brisbin et al. 2008). As a first line of defense against pathogens, the avian gut is coated with mucus layer (Forder et al. 2012) consisting of a loose outer layer that harbors microbiota and an inner compact layer which repels most bacteria (Hansson and Johansson 2010). Recent studies suggest that components of the avian mucus modulate the expression of critical virulence traits of pathogens. *C. jejuni* colonizes the chicken gut in high numbers yet does not cause any disease in birds. In vitro data suggest that the presence of chicken mucin reduces the attachment and invasion efficiency of *C. jejuni* to intestinal epithelial cells (Byrne et al. 2007; Alemka et al. 2010, 2012). Struwe et al. (2015) used liquid chromatography mass spectrometry to perform structural analysis of O-glycans released in chicken intestinal mucin. The O-glycans were abundantly sulfated compared with the human intestinal mucus samples. In addition, alpha 1–2 linked fucose residues, which have high binding affinity to *C. jejuni*, were identified in the small and large intestines. These variations suggest that chicken gastrointestinal tract has evolved to support the colonization of *C. jejuni*. Moreover, N-linked glycosylation of surface proteins in *C. jejuni* enhances its fitness by protecting bacterial proteins from gut protease cleavage (Alemka et al. 2013).

Another critical component of innate immune system active in the poultry gut is antimicrobial peptides (AMP) present on the intestinal epithelial surface (Brisbin et al. 2008). Antimicrobial peptides have been described as a host defense that has coevolved with microbes (Zasloff 2002). Produced by all major eukaryotes, AMP provide immediate, effective, and non-specific defense against infections by bacterial, viral, or fungal organisms. Based on their secondary structure, these small molecules (15–50 amino acids) are classified into four major classes, namely, alpha helix, beta-sheet, and extended and loop peptides (Lai et al. 2009). Antimicrobial peptides act primarily by damaging the bacterial membrane (Shai 1999; Yang et al. 2001). Other mechanisms include suppression of protein, nucleic acid, or cell wall synthesis and inhibition of enzymatic activity (Brogden 2005). These attributes make them attractive candidates for the design of new antimicrobial agents. In poultry, beta-defensins are the well-characterized antimicrobial peptides that are produced by avian epithelial cells, macrophages, and heterophils (Jenssen et al. 2006; Derache et al. 2009). Brisbin et al. (2008) showed that infection with *Salmonella* resulted in an increase in the expression of beta-defensin genes in chickens. Ebers et al. (2009) profiled the expression of avian beta-defensin genes in chicken oviduct epithelial cells before and after infection with *S. Enteritidis*, where the pathogen was found to modulate the expression of select defensin genes. Moreover, the *pipB* mutant elicited significantly higher levels of avian beta-defensins 2 and 8, suggesting that the T3SS-2 effector protein PipB plays a role in dampening the beta-defensin-based innate immunity in birds during *Salmonella* invasion of chicken oviduct epithelial cells.

The cellular components of the avian immune system such as macrophages and heterophils also confer protection from enteric infection and are recruited to the site of infection to kill invading pathogens (Brisbin et al. 2008). Meade et al. (2009) studied the early host immune response to *Salmonella* and *Campylobacter jejuni* colonization in chickens. *Salmonella* infection induced significant changes in circulating heterophil and monocyte/macrophage populations, while *C. jejuni* infection increased monocyte/macrophage populations. Toll-like receptor 1 (TLR1) gene expression was decreased by *Salmonella*; however, beta-defensin genes (AvBD3, AvBD10, AvBD12) were significantly increased. In contrast, *Campylobacter* infection induced an increase in TLR21 expression but significantly reduced the expression of AMP genes (AvBD 3, AvBD 4, AvBD 8, AvBD 13, AvBD 14). Enteric pathogens have evolved to utilize some of the physiological changes in the gut to their advantage. For example, pathogen colonization of avian gut leads to mild inflammation with influx of macrophages and heterophils to the lamina propria and villus epithelium (Crhanova et al. 2011). *Salmonella* has developed mechanisms to survive in host cells such as macrophages which help in its systemic dissemination (Buchmeier and Heffron 1991; Cirillo et al. 1998; Malik-Kale et al. 2011; Swart and Hensel 2012). More recently, studies suggest that *S. Enteritidis* and *S. Typhimurium* can induce mild inflammation leading to influx of macrophages and heterophils to villus epithelium that aids in their systemic dissemination (Fasina et al. 2010; Van Immerseel et al. 2002). The major virulence factors participating in this pathogenesis and intracellular survival have been characterized (Ibarra and Steele-Mortimer 2009). Type III secretion systems, LPS, peptidoglycan, and flagellin that trigger inflammatory response through pathogen-associated molecular patterns and pro-inflammatory cytokines are some of the major virulence factors responsible for epithelial cell invasion and intracellular survival in macrophages (Ahmer and Gunn 2011; Zhou and Galán 2001; Abrahams and Hensel 2006). Taken together, these results suggest that the innate immune system of poultry responds differently to *Salmonella* and *Campylobacter* challenge and pathogens have evolved with strategies that facilitate their survival in the gut.

The interactions of host gut microbiota and innate immune response also trigger adaptive immune response activation (Palm et al. 2015; Lee and Mazmanian 2010). Studies in mice have revealed that a lack of adaptive immune system leads to alterations in the gut microbiota composition (Kato et al. 2014; Kawamoto et al. 2014). In the poultry gut, the B and T cells are found in organized lymphoid tissues (e.g., bursa of Fabricius, cecal tonsils, Peyer's patches) and some in lamina propria and epithelium (Brisbin et al. 2008; Bar-Shira et al. 2003). These cells primarily contribute to adaptive immunity through antibody-mediated and cell-mediated responses, respectively. Several researchers have investigated the effect of microbiome modulations on the adaptive immune response of chickens. Kim et al. (2010) investigated the effect of dietary plant-derived phytochemicals on the translational regulation of genes associated with immune modulation. Many of the genes contributing to metabolism, immunity, antigen presentation, and inflammatory response were modulated by the phytochemicals such as capsicum oleoresin and cinnamaldehyde. In another study, Du et al. (2016) tested the effects of thymol and carvacrol

on intestinal integrity and immune responses of broiler chickens challenged with *Clostridium perfringens*. Results revealed an increase in interleukin-1-beta and TLR2 mRNA expression. Moreover, the expression of secretory IgA was also upregulated in response to pathogen challenge. Interestingly, expression of claudin-1 and occludin mRNA (responsible for intestinal integrity) was downregulated. Dietary essential oil alleviated gut lesions and increased expression of occludin mRNA, suggesting that they modulate immune responses in *C. perfringens*-challenged broiler chickens. In addition to phytochemicals, probiotics have also been tested for their role as immune-modulators in chickens. Chickens receiving probiotics containing *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Streptococcus faecalis* showed increased systemic antibody reactive to tetanus toxoid and *C. perfringens* alpha toxin (Haghighi et al. 2006). In addition, intestinal IgG and serum IgG and IgM were also increased in chickens supplemented with the probiotics, suggesting the induction of natural antibodies for maintaining chicken health. Similar results have been reported with other groups of lactobacilli (Koenen et al. 2004; Brisbin et al. 2012). However, the exact mechanism(s) by which probiotic bacteria bring about aforementioned immune modulations is not clear and requires further research.

10.3.4 Pathogen Colonization

As discussed previously, the intestinal microbiota interacts with the gut mucosal immune system to maintain homeostasis, and disruption of this interaction leads to disease state. Perumbakkam et al. (2014) investigated the effect of Marek's disease virus on core gut microbiota of chickens. The virus targets lymphoid tissue such as the bursa of Fabricius, thymus, and spleen; however, limited literature exists on its interaction with gut-associated lymphoid tissue. The viral infection altered the core cecal microbiota early after infection (2–7 days) and in the late phase of infection (28–35 days) suggesting a correlation between viral infection and microbial composition of the intestinal tract. Necrotic enteritis caused by *C. perfringens* is another disease that affects poultry globally. Some of the factors that predispose the gastrointestinal environment for *C. perfringens* colonization include high levels of nonstarch polysaccharides, high protein fishmeal, and factors that induce epithelial cell damage (*Fusarium* mycotoxins, *Eimeria* infection). Recent studies suggest that the onset of necrotic enteritis is associated with changes in gut microbiota, including shifts in the alpha and beta diversity (Stanley et al. 2012, 2014). Butyrate-producing strains of *Ruminococcaceae* and *Lachnospiraceae* decrease with an increase in protein content of feed or *Eimeria* infection (Wu et al. 2014). Butyrate stabilizes intestinal integrity through its anti-inflammatory action (Eeckhaut et al. 2011) and activation of glucagon-like peptide 2(GLP-2). The GLP-2 hormone induces cytokine production that improves tight junction-based intestinal integrity (Hiramatsu et al. 2005). Pathogens such as *Campylobacter* and *Salmonella* which colonize chickens in high numbers tend to achieve this without perturbing the microbiome

significantly. Thibodeau et al. (2015) observed that the alpha diversity was conserved, while beta diversity was moderately affected during high cecal colonization by *C. jejuni*. Similar effects were observed by Videnska et al. (2013) with *S. Enteritidis* colonization in young chickens. The efficacy of trans-cinnamaldehyde and caprylic acid in reducing *S. Enteritidis* colonization in 24- and 40-week-old layer chickens and corresponding shifts in the microbiome was investigated (Upadhyaya et al. 2015a). Results revealed that the phytochemicals (caprylic acid 0.7, 1%; trans-cinnamaldehyde 1, 1.5%) were effective in reducing *S. Enteritidis* in the cecum, on the eggshell and in the yolk. There was no change in the alpha diversity, beta diversity, and major bacterial phylotypes (*Firmicutes*, *Bacteroides*, and *Proteobacteria*) across treatments and time (days 0, 1, 7, 10, 20, 30, 60). Overall, these results suggest that pathogen colonization in chickens usually occurs without major shifts in the microbiota.

10.4 Applications of Transcriptomics and Proteomics to Study Poultry Gut Microbiota Function

After characterizing the gut microbiota, it is critical to delineate the underlying mechanisms by which they impact gut physiology and health of the host. Technological advancements in RNAseq have enabled us to study genes that are actively expressed in a complex bacterial community such as the gut microbiota. This facilitates gaining insight into microbial function, interactions with the host, and changes that occur during disease state. Several researchers investigated the transcriptomic profile of gut microbiota, enteric pathogens, and poultry host to develop an understanding of various host-pathogen interactions. Taveirne et al. (2013) used RNAseq to study the complete *C. jejuni* transcriptome during colonization in chickens. A total of 272 genes that are differentially expressed during chicken colonization were identified. Some of the *C. jejuni* genes that were increased in abundance include genes coding for transport (pstSAC, ChuABCD), stress response (katA, cgb), and energy metabolism (dsbAB, sulphite oxidase, cytochrome c family). In addition to differential gene expression, several noncoding RNAs were also identified that are likely induced due to stress or nutrient limitation and potentially contribute to chicken colonization.

Li et al. (2010) investigated the cecal response of two genetic lines of chickens with different susceptibility to *C. jejuni* colonization. The more resistant line A birds responded by an upregulation of lymphocyte activation and increased expression of oligomerization domain (NOD)-like receptor. Also known as NALP1, these proteins function as sensors for detecting microbial components in the host cell similar to the role of toll-like receptors. In another study, Matulova et al. (2012) studied the changes in spleen transcriptome after infection with *S. Enteritidis* to identify potential markers of infection. A total of 40 genes were upregulated. Genes coding for avidin, immune responsive gene (IRG1), fatty acid binding protein (EXFABP), chemokine ah221, and trappin-6-like protein (TRAP6) were some of the upregulated genes.

Complementary DNA sorting revealed that the abovementioned genes were preferentially expressed in the macrophages. Furthermore, some of the abovementioned genes (EXFABP, IRG1, TRAP6, AH221) were also induced in the cecum of infected birds on day 1 post challenge. Ciraci et al. (2010) studied the genome-wide transcriptome profile of chicken macrophages exposed to *S. Typhimurium*-798 derived endotoxins. Pathway analysis showed that 10% of total differentially expressed genes were involved in inflammatory response. Endotoxin exposure significantly affected the mRNA expression of IL1B, IL6, IL8, and TLR15 in chicken macrophages (HD11). Overall, these studies provide insights into the expression of key genes during host-pathogen-microbiota interplay in the gut.

A major challenge while using DNA-based approaches for characterizing microbiota functionality is that the data predict potential functions based on the presence of certain genes. In addition, questions such as expression levels of genes and source of DNA (live/dead cells) require additional experimentation. However, such limitations can be addressed using proteomics as a tool for characterizing microbiota function. High-throughput metaproteomic analysis provides a clear fingerprint of the metabolic state of a microbial community such as in the gut (Verberkmoes et al. 2008) and is a useful resource for providing meaningful data on host-microbiota interactions and microbiota function. Since the identified proteins can be assigned to taxa as well as functions, it is very useful to study different functional properties of a microbial community, including any posttranslational modifications. Tang et al. (2014) conducted a metaproteomic analysis of fecal samples to study the adaptation process of chicken gut microbiota. Metaproteomic analysis identified 3673 proteins of which 380 proteins belonged to *Lactobacillus* spp., 155 to *Clostridium* spp., and 66 to *Streptococcus* spp. The most frequently identified proteins were chaperon proteins (GroEL, DnaK), dehydrogenases, elongation factor proteins, heat shock chaperones, and pyruvate kinases. In addition to studying the metaproteome, researchers have also investigated the proteome of a poultry enteric pathogen as well as the host response. Upadhyay et al. (2017a) investigated the effect of trans-cinnamaldehyde (essential oil obtained from cinnamon bark) on the proteome of *C. jejuni*. Results revealed that trans-cinnamaldehyde downregulated the expression of several proteins (AspA, FrdA, AhpC, PstS, CeuE, HemC) critical for aero tolerance, acid tolerance, stress response, and colonization in chickens. Follow-up investigation revealed that the phytochemical was able to significantly reduce *C. jejuni* colonization in broiler chickens (Upadhyay et al. 2017b). In another investigation, Upadhyaya et al. (2017) studied the changes in *C. jejuni* proteome in response to subinhibitory concentrations of eugenol (essential oil obtained from cloves). Interestingly, the group of proteins downregulated by eugenol (PorA, CadF, CheA, CheV, CheY, LuxS, TatA, TatB, MotA, MotB) primarily contribute to bacterial adhesion, locomotion, and cell-to-cell communication, and they were different from those observed with trans-cinnamaldehyde, suggesting that the two essential oils work via different mechanisms/pathways. O'Reilly et al. (2017) investigated changes in chicken intestinal proteome in response to microbial challenge and age of birds and observed significant changes in the small intestinal proteome sampled from 12 to 22 days of age in chickens. Proteins such as actin and

actin-associated proteins increased over time. Villin-1, an actin-associated anti-apoptotic protein, was reduced in abundance in birds challenged with *C. jejuni* and coccidial oocysts, indicating that many of the changes in cytoskeletal protein abundance in the challenged birds were because of an increased rate of apoptosis. Several heat shock proteins also decreased over time, especially in challenged birds. Some of the challenges that still need further research include (1) high complexity of the miscrobial community, (2) low coverage of the complete proteome by existing technology, and (3) high sequence similarity between many proteins, especially those that perform similar functions (Haange and Jehmlich 2016). One potential approach that is being employed is the use of gnotobiotic animal models that have a relatively simple microbiota consisting of only well-characterized species. This may facilitate greater protein coverage during analysis and enable better interpretations (Woting et al. 2014).

10.5 Applications of Metabolomics to Study Host-Microbiota Interactions

The metabolome is the final transcript of the genome that consists of all low molecular weight compounds (metabolites; less than 1500 Da) in a cell, tissue, or organism. The metabolites produced by the microbiota are key signaling compounds that, along with proteins, form the biochemical basis of cross talk with other microorganisms and hosts. Metabolomics is a powerful scientific approach that can be used to study such metabolite-based chemical cross talk. More recently, metabolomics is being extensively employed to study gut physiology in health and disease (Holmes et al. 2011), identify biomarkers for rapid diagnosis of a physiological state (Dunn and Ellis 2005), and characterize microbial metabolism (Vaidyanathan et al. 2006).

10.5.1 Analytical Tools to Study Microbial Metabolomics

Metabolomic methodologies fall into two broad categories: untargeted metabolomics, a comprehensive analysis of all measurable compounds in a sample and targeted metabolomics, and the measurement of defined classes of well-characterized and annotated metabolites (Roberts et al. 2012). With the rapid development of a plethora of analytical platforms, we can effectively detect and quantify metabolites and characterize relevant metabolic pathways. Some of the popular analytical platforms include liquid and gas chromatography (LC, GC), high-pressure and ultra-pressure liquid chromatography (HPLC, UPLC), Fourier transform infrared spectroscopy (FTIR) coupled with mass spectrometry (MS), and nuclear magnetic resonance spectroscopy (NMR) (Zheng et al. 2011; Vernocchi et al. 2012, 2016). A brief description of these methods is presented in this section. Additional details are described elsewhere (Roberts et al. 2012; Vernocchi et al. 2016).

10.5.1.1 Gas Chromatography Mass Spectrometry (GC-MS)

Gas chromatography mass spectrometry (GC-MS) is the gold standard in metabolomics (Harrigan and Goodacre 2012) that is used to study heat stable and volatile compounds such as alcohols, esters, aldehydes, ketones, and fatty acids. The metabolites are separated by GC followed by detection by electron-impact (EI) mass spectrometer. The samples are prepared by liquid/solid phase extraction or by headspace-solid phase microextraction based on specific requirements (Dettmer et al. 2007; Pawliszyn 1997). The metabolites are stabilized by a two-stage derivatization process (Roessner et al. 2000). Metabolite quantification is conducted by external calibration or response ratio (peak area of test metabolite/peak area of internal standard). Identification of metabolites is conducted by matching retention time and mass spectrum of the sample peak with a pure compound previously analyzed under identical conditions (Fiehn et al. 2000) or against a commercial database.

10.5.1.2 Liquid Chromatography Mass Spectrometry (LC-MS)

Liquid chromatography mass spectrometry is an analytical technique with a wide range of applications in biotechnology, food, pharmaceutical, and cosmetic industry. The LC-based metabolite separation is followed by electrospray ionization (ESI) or atmospheric pressure chemical ionization (Bakhtiar et al. 2004). The combination of LC with MS permits analysis of polar, nonpolar, and neutral compounds. Unlike GC-MS, the LC-MS technique does not require sample volatility or sample derivatization, thereby facilitating accessibility to much greater mass ranges than permitted by GC-MS. Metabolite quantification is obtained by external calibration/response ratio. Moreover, ESI does not provide direct metabolite identification due to lack of molecular ion fragmentation and mass spectral libraries. With the development of HPLC and UPLC, the analysis time has been shortened along with higher resolution and sensitivity (Smirnov et al. 2016).

10.5.1.3 Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectroscopy studies the vibrational properties of compounds based on the presence of functional groups in their structure (Berthomieu and Hienerwadel 2009). These functional group-specific IR signatures are often used to identify the metabolites in a sample. Although comparatively insensitive as compared to GC/LC-MS, this technique allows high-throughput screening and classification of biological samples (Ellis and Goodacre 2001). It has been used to detect spoilage in meat (Ellis and Goodacre 2001), milk (Nicolaou and Goodacre 2008), and strawberries (Dong et al. 2013) and to detect the bovine mastitis marker (Schabauer et al. 2014) and characterization of food spoilage fungi (Shapaval et al. 2013).

10.5.1.4 Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance refers to the phenomenon in which nuclei in the presence of a magnetic field absorb energy leading to a high-energy state followed by release of the energy at a specific resonance frequency. This energy is quantified using a spectrometer. NMR spectroscopy is used to study the structure of molecules, intermolecular interactions, composition of biological mixtures and metabolites. Some of the advantages of using NMR spectroscopy include (a) simpler sample preparation, (b) identification of compounds with identical masses and low ionization state, and (c) nondestructive nature (Lenz et al. 2004; Smolinska et al. 2012). Moreover, site-specific NMR imaging offers strategies for metabolic investigations in live animals (Markley et al. 2017). Some of the disadvantages of NMR spectroscopy include low sensitivity and restricted annotation capability (Jansson et al. 2009). Le Roy et al. (2016) used NMR spectroscopy to characterize chicken tissues (liver, kidney, spleen, egg yolk, plasma, colon, cecum, fecal water, pectoral muscle, brain) followed by metabolite identification. Approximately 80 metabolites were identified to develop the first chicken metabolome atlas. Only eight metabolites were found to be common across all tissue samples. In another study, Quirk et al. (1989) studied the metabolites present in chicken small intestinal cells using NMR spectroscopy. High concentrations of serine ethanolamine phosphodiester (SEP), creatine, aurine, and acidic amino acids were found in all segments of the intestinal tract. Taurine (~8 mM), choline (0.5 mM), and betaine (~0.5 mM) were evenly distributed throughout the segments. These works constitute development of a database for future NMR-based metabolomic investigations in relation to poultry production and health.

10.5.2 Microbial Metabolites

Several metabolites are produced by the gut microbiota that facilitates host-microbiota cross talk. Short-chain fatty acids are produced as a result of fermentation of indigestible polysaccharides, fiber, and proteins (Arora and Sharma 2011). Bacterial groups such as *Lactobacillus*, *Actinobacteria* (*Bifidobacterium* spp.), and *Clostridium* clusters IV and XIVa play a major role in SCFA synthesis and metabolism (Nicholson et al. 2012). Acetate is an important SCFA that facilitates development of colonic epithelium through its trophic effect. It is also absorbed by tissues participating in cholesterol synthesis (Scheppach et al. 1991). Propionate, on the other hand, inhibits cholesterol synthesis (Scheppach 1994), and the ratio of acetate to propionate is used as an index for determining the risk of cardiovascular disease in humans (Wong et al. 2006). Butyrate represents the major energy source for distal gut and nourishes the colonic mucosa (Walton et al. 2013). The production of SCFA in poultry gut has been studied to elucidate their association with health and productivity. Chang et al. (2016) studied the beneficial effect of *Bidens pilosa* (flowering plant in aster family; commonly known as beggartick or Spanish

needle) on body weight gain, FCR, gut microbiota composition, and susceptibility to coccidiosis in chickens. Results revealed that administration of *Bidens pilosa* significantly elevated body weight gain and reduced feed conversion ratio. Metagenomic analysis revealed an increase in probiotic genera such as *Alistipes*, *Bacteroides*, *Lactobacillus*, and *Ruminococcus*. *Bacteroides* and *Ruminococcus* have been previously reported to be involved in polysaccharide degradation and production of propionate in chicken gut (Sergeant et al. 2014). Vitamins are another group of critical micronutrients that play an essential role in biochemical reactions/pathways in the majority of animal hosts. *Bacteroides* and *Lactobacillus* were reported to produce essential vitamins such as vitamins K and B12 and folic acid (Luo et al. 2003). These results suggest that the beneficial effects observed with *Bidens pilosa* could be partially due to production of useful metabolites such as SCFA and vitamins.

In addition to SCFA and vitamins, other microbiota-transformed compounds include bile salts and polyphenols (Vernocchi et al. 2016). The metabolism of bile salts is primarily associated with bacterial genera such as *Bacteroides*, *Clostridium*, *Lactobacillus*, *Bifidobacterium*, and *Enterobacter* (Ridlon et al. 2006). In poultry, bile salts contribute to lipid digestibility and weight gain, and dietary interventions that modulate bile salts levels in the gut also affect digestibility and poultry health. Maisonnier et al. (2003) studied the effect of infeed supplementation of guar gum on lipid digestibility, intestinal bile salts levels, and health in broiler chickens. Results revealed that guar gum reduced bile salts levels, thereby leading to altered lipid digestibility and reduced weight gain in birds. Polyphenols are plant nutraceuticals that are considered as bioactive components in the diet (Manach et al. 2004). They have high structural diversity; however, most of them occur as glycosylated derivatives and require intestinal transformation through host enzymes or gut microbiota-mediated metabolism (Marin et al. 2015). Major groups of polyphenols include tannins, flavonoids, chlorogenic acids, and coumarins. Several investigations have studied the interaction of polyphenols with the intestinal microbiota in mice (Duda-Chodak et al. 2015; Ozdal et al. 2016) and humans (Van Duynhoven et al. 2011). In addition, the role of several polyphenols as effective antimicrobials for controlling foodborne pathogens in chickens has been investigated (Upadhyaya et al. 2015b, c; Kollanoor-Johny et al. 2012). However, studies investigating the metabolism of polyphenols including their absorption kinetics in chickens require further research.

10.6 Conclusion

Scientific advancements, especially in genomics, transcriptomics, and metabolomics, have contributed to rapidly accumulating knowledge in gut health. Several studies have elucidated the connection between microbial metagenome, metatranscriptome, proteome, and metabolome in relation to poultry health, productivity, and safety. As new research delineates the complete gut metabolome in various

physiological states (health and disease), a better understanding of host response to various environmental cues, microbiota, and chemicals would possibly emerge. The role of microbial metabolites in epigenetic activation/repression of gene expression through posttranslational and posttranscriptional modifications is a relatively less explored field that holds promise for new discoveries in gut health and productivity in poultry production.

References

Abbas Hilmi, H., Surakka, A., Apajalahti, J., & Saris, P. E. J. (2007). Identification of the most abundant lactobacillus species in the crop of 1- and 5-week-old broiler chickens. *Applied and Environmental Microbiology, 73*, 7867–7873.

Abrahams, G. L., & Hensel, M. (2006). Manipulating cellular transport and immune responses: Dynamic interactions between intracellular *Salmonella enterica* and its host cells. *Cellular Microbiology, 8*(5), 728–737.

Ahmer, B. M., & Gunn, J. S. (2011). Interaction of *Salmonella* spp. with the intestinal microbiota. *Frontiers in Microbiology, 2*, 101.

Alemka, A., Corcionivoschi, N., & Bourke, B. (2012). Defense and adaptation: The complex inter-relationship between *Campylobacter jejuni* and mucus. *Frontiers in Cellular and Infection Microbiology, 2*, 15.

Alemka, A., Nothaft, H., Zheng, J., & Szymanski, C. M. (2013). N-glycosylation of *Campylobacter jejuni* surface proteins promotes bacterial fitness. *Infection and Immunity, 81*(5), 1674–1682.

Alemka, A., Whelan, S., Gough, R., Clyne, M., Gallagher, M. E., Carrington, S. D., et al. (2010). Purified chicken intestinal mucin attenuates *Campylobacter jejuni* pathogenicity in vitro. *Journal of Medical Microbiology, 59*(8), 898–903.

Amit-Romach, E., Sklan, D., & Uni, Z. (2004). Microflora ecology of the chicken intestine using 16S ribosomal DNA primers. *Poultry Science, 83*(7), 1093–1098.

Angelakis, E., Armougom, F., Million, M., & Raoult, D. (2012). The relationship between gut microbiota and weight gain in humans. *Future Microbiology, 7*(1), 91–109.

Anonymous. (2005). Centers for Disease Control and Prevention, Preliminary foodnet data on the incidence of infection with pathogens transmitted commonly through food—10 states, United States, 2005. *Morbidity and Mortality Weekly Report, 55*, 392–395.

Apajalahti, J., & Kettunen, A. (2006). Microbes of the chicken gastrointestinal tract. In G. C. Perry (Ed.), *Avian gut function in health and disease* (Poultry Science Symposium Series) (Vol. 28, pp. 121–113). Wallingford: CABI Publishing.

Apajalahti, J. H., & Bedford, M. R. (1999). Improve bird performance by feeding its microflora. *World's Poultry Science Journal, 15*, 20–23.

Arora, T., & Sharma, R. (2011). Fermentation potential of the gut microbiome: Implications for energy homeostasis and weight management. *Nutrition Reviews, 69*(2), 99–106.

Bakhtiar, R., Ramos, L., & Tse, F. L. (2004). Toxicokinetic assessment of methylphenidate (Ritalin®) in a 13-week oral toxicity study in dogs. *Biomedical Chromatography, 8*(1), 45–50.

Bakshi, C. S., Singh, V. P., Malik, M., Singh, R. K., & Sharma, B. (2003). 55 kb plasmid and virulence-associated genes are positively correlated with *Salmonella enteritidis* pathogenicity in mice and chickens. *Veterinary Research Communications, 27*, 425–432.

Bar-Shira, E., Sklan, D., & Friedman, A. (2003). Establishment of immune competence in the avian GALT during the immediate post-hatch period. *Developmental and Comparative Immunology, 27*(2), 147–157.

Belkaid, Y., & Hand, T. W. (2014). Role of the microbiota in immunity and inflammation. *Cell, 157*(1), 121–141.

Berthomieu, C., & Hienerwadel, R. (2009). Fourier transform infrared (FTIR) spectroscopy. *Photosynthesis Research, 101*(2–3), 157–170.

Bjerrum, L., Engberg, R. M., Leser, T. D., Jensen, B. B., Finster, K., & Pedersen, K. (2006). Microbial Community composition of the ileum and cecum of broiler chickens as revealed by molecular culture-based techniques. *Poultry Science, 85*, 1151–1164.

Bregendahl, K., & Roberts, S. (2006, March 21–23). *Nutritional strategies to reduce ammonia emissions from laying hens*. In Proc. Midwest Poultry Federation Convention, St. Paul, MN.

Brisbin, J. T., Gong, J., & Sharif, S. (2008). Interactions between commensal bacteria and the gut-associated immune system of the chicken. *Animal Health Research Reviews, 9*(1), 101–110.

Brisbin, J. T., Parvizi, P., & Sharif, S. (2012). Differential cytokine expression in T-cell subsets of chicken cecal tonsils co-cultured with three species of *Lactobacillus*. *Beneficial Microbes, 3*(3), 205–210.

Brogden, K. A. (2005). Antimicrobial peptides: Pore formers or metabolic inhibitors in bacteria? *Nature Reviews. Microbiology, 3*(3), 238.

Buchmeier, N. A., & Heffron, F. (1991). Inhibition of macrophage phagosome-lysosome fusion by *Salmonella* Typhimurium. *Infection and Immunity, 59*(7), 2232–2238.

Byrne, C. M., Clyne, M., & Bourke, B. (2007). *Campylobacter jejuni* adhere to and invade chicken intestinal epithelial cells in vitro. *Microbiology, 153*(2), 561–569.

Cagri, A., Ustunol, Z., & Ryser, E. T. (2004). Antimicrobial edible films and coatings. *Journal of Food Protection, 67*(4), 833–848.

Centers for Disease Control and Prevention. (2014). Incidence and trends of infection with pathogens transmitted commonly through food – foodborne diseases active surveillance network, 10 U.S. sites, 2006–2013. *Morbidity and Mortality Weekly Report, 63*, 328–332.

Chambers, J. R., & Gong, J. (2011). The intestinal microbiota and its modulation for *Salmonella* control in chickens. *Food Research International, 44*(10), 3149–3159.

Chang, C. L., Chung, C. Y., Kuo, C. H., Kuo, T. F., Yang, C. W., & Yang, W. C. (2016). Beneficial effect of *Bidens pilosa* on body weight gain, food conversion ratio, gut bacteria and coccidiosis in chickens. *PLoS One, 11*(1), 0146141.

Chapman, H. D., & Johnson, Z. B. (2002). Use of antibiotics and roxarsone in broiler chickens in the USA: Analysis for the years 1995 to 2000. *Poultry Science, 81*, 356–364.

Chow, J., & Mazmanian, S. K. (2010). A pathobiont of the microbiota balances host colonization and intestinal inflammation. *Cell Host & Microbe, 7*(4), 265–276.

Ciraci, C., Tuggle, C. K., Wannemuehler, M. J., Nettleton, D., & Lamont, S. J. (2010). Unique genome-wide transcriptome profiles of chicken macrophages exposed to *Salmonella*-derived endotoxin. *BMC Genetics, 11*(1), 545.

Cirillo, D. M., Valdivia, R. H., Monack, D. M., & Falkow, S. (1998). Macrophage-dependent induction of the *Salmonella* pathogenicity island 2 type III secretion system and its role in intracellular survival. *Molecular Microbiology, 30*(1), 175–188.

Clench, M. H., & Mathias, J. R. (1995). The avian cecum: A review. *The Wilson Bullet, 107*(1), 93–121.

Costa, M. C., Bessegatto, J. A., Alfieri, A. A., Weese, J. S., João Filho, A. B., & Oba, A. (2017). Different antibiotic growth promoters induce specific changes in the cecal microbiota membership of broiler chicken. *PLoS One, 12*(2), 0171642.

Crhanova, M., Hradecka, H., Faldynova, M., Matulova, M., Havlickova, H., Sisak, F., et al. (2011). Immune response of chicken gut to natural colonization by gut microflora and to *Salmonella enterica* serovar Enteritidis infection. *Infection and Immunity, 79*(7), 2755–2763.

Danzeisen, J. L., Kim, H. B., Isaacson, R. E., Tu, Z. J., & Johnson, T. J. (2011). Modulations of the chicken cecal microbiome and metagenome in response to anticoccidial and growth promoter treatment. *PLoS One, 6*(11), 27949.

Derache, C., Labas, V., Aucagne, V., Meudal, H., Landon, C., Delmas, A. F., et al. (2009). Primary structure and antibacterial activity of chicken bone marrow-derived β-defensins. *Antimicrobial Agents and Chemotherapy, 53*(11), 4647–4655.

Dettmer, K., Aronov, P. A., & Hammock, B. D. (2007). Mass spectrometry-based metabolomics. *Mass Spectrometry Reviews, 26*(1), 51–78.

Dibner, J. J., & Richards, J. D. (2005). Antibiotic growth promoters in agriculture: History and mode of action. *Poultry Science, 84*, 634–643.

Ding, J., Zhao, L., Wang, L., Zhao, W., Zhai, Z., Leng, L., et al. (2016). Divergent selection-induced obesity alters the composition and functional pathways of chicken gut microbiota. *Genetics Selection Evolution, 48*(1), 93.

Donaldson, G. P., Lee, S. M., & Mazmanian, S. K. (2016). Gut biogeography of the bacterial microbiota. *Nature Reviews Microbiology, 14*(1), 20–32.

Dong, J., Zhang, Y., Tang, X., Jin, W., & Han, Z. (2013). Differences in volatile ester composition between Fragaria× ananassa and F. vesca and implications for strawberry aroma patterns. *Scientia Horticulturae, 150*, 47–53.

Donohoe, D. R., Garge, N., Zhang, X., Sun, W., O'Connell, T. M., Bunger, M. K., et al. (2011). The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metabolism, 13*(5), 517–526.

Du, E., Wang, W., Gan, L., Li, Z., Guo, S., & Guo, Y. (2016). Effects of thymol and carvacrol supplementation on intestinal integrity and immune responses of broiler chickens challenged with *Clostridium perfringens*. *Journal of Animal Science and Biotechnology, 7*(1), 9.

Duda-Chodak, A., Tarko, T., Satora, P., & Sroka, P. (2015). Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: A review. *European Journal of Nutrition, 54*(3), 325–341.

Dumonceaux, T. J., Hill, J. E., Hemmingsen, S. M., & Van Kessel, A. G. (2006). Characterization of intestinal microbiota and response to dietary virginiamycin supplementation in the broiler chicken. *Applied and Environmental Microbiology, 72*(4), 2815–2823.

Dunkley, K. D., Dunkley, C. S., Njongmeta, N. L., Callaway, T. R., Hume, M. E., Kubena, L. F., et al. (2007). Comparison of in vitro fermentation and molecular microbial profiles of high-fiber feed substrates incubated with chicken cecal inocula. *Poultry Science, 86*(5), 801–810.

Dunn, W. B., & Ellis, D. I. (2005). Metabolomics: Current analytical platforms and methodologies. *TrAC Trends in Analytical Chemistry, 24*(4), 285–294.

Ebers, K. L., Zhang, C. Y., Zhang, M. Z., Bailey, R. H., & Zhang, S. (2009). Transcriptional profiling avian beta-defensins in chicken oviduct epithelial cells before and after infection with *Salmonella enterica* serovar Enteritidis. *BMC Microbiology, 9*(1), 153.

Eeckhaut, V., Van Immerseel, F., Croubels, S., De Baere, S., Haesebrouck, F., Ducatelle, R., et al. (2011). Butyrate production in phylogenetically diverse Firmicutes isolated from the chicken caecum. *Microbial Biotechnology, 4*(4), 503–512.

Ellis, D. I., & Goodacre, R. (2001). Rapid and quantitative detection of the microbial spoilage of muscle foods: Current status and future trends. *Trends in Food Science & Technology, 12*(11), 414–424.

Emmerson, D. A. (1997). Commercial approaches to genetic selection for growth and feed conversion in domestic poultry. *Poultry Science, 76*(8), 1121–1125.

Fasina, Y. O., Bowers, J. B., Hess, J. B., & McKee, S. R. (2010). Effect of dietary glutamine supplementation on *Salmonella* colonization in the ceca of young broiler chicks. *Poultry Science, 89*(5), 1042–1048.

Fiehn, O., Kopka, J., Dörmann, P., Altmann, T., Trethewey, R. N., & Willmitzer, L. (2000). Metabolite profiling for plant functional genomics. *Nature Biotechnology, 18*(11), 1157.

Foley, S. L., Nayak, R., Hanning, I. B., Johnson, T. J., Han, J., & Ricke, S. C. (2011). Population dynamics of *Salmonella enterica* serotypes in commercial egg and poultry production. *Applied and Environmental Microbiology, 77*(13), 4273–4279.

Forder, R. E. A., Nattrass, G. S., Geier, M. S., Hughes, R. J., & Hynd, P. I. (2012). Quantitative analyses of genes associated with mucin synthesis of broiler chickens with induced necrotic enteritis. *Poultry Science, 91*(6), 1335–1341.

Frost, A. J., & Woolcock, J. B. (1991). Antibiotics and animal production. In J. B. Woolcock (Ed.), *Microbiology of animals and animal products* (pp. 181–194). New York: Elsevier.

Gillespie, I. A., O'Brien, S. J., Adak, G. K., Tam, C. C., Frost, J. A., Bolton, F. J., et al. (2003). Point source outbreaks of *Campylobacter jejuni* infection--are they more common than we think and what might cause them? *Epidemiology and Infection, 130*(3), 367.

Gong, J., Si, W., Forster, R. J., Huang, R., Yu, H., Yin, Y., et al. (2006). 16S rRNA gene-based analysis of mucosa-associated bacterial community and phylogeny in the chicken gastrointestinal tracts: From crops to ceca. *FEMS Microbiology Ecology, 59*(1), 147–157.

Grover, M., & Kashyap, P. C. (2014). Germ-free mice as a model to study effect of gut microbiota on host physiology. *Neurogastroenterology and Motility, 26*(6), 745–748.

Haange, S. B., & Jehmlich, N. (2016). Proteomic interrogation of the gut microbiota: Potential clinical impact. *Expert Review of Proteomics, 13*(6), 535–537.

Haghighi, H. R., Gong, J., Gyles, C. L., Hayes, M. A., Zhou, H., Sanei, B., et al. (2006). Probiotics stimulate production of natural antibodies in chickens. *Clinical and Vaccine Immunology, 13*(9), 975–980.

Hansson, G. C., & Johansson, M. E. (2010). The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Gut Microbes, 1*(1), 51–54.

Harrigan, G. G., & Goodacre, R. (Eds.). (2012). *Metabolic profiling: Its role in biomarker discovery and gene function analysis*. New York: Springer Science.

Hiramatsu, K., Yamasaki, A. & Shioji, T. (2005). Immunohistochemical and morphometrical studies on the distribution of glucagon-like peptide-1 (GLP-1)- immunoreactive cells in the chicken intestine. *The Journal of Poultry Science, 42*, 223–229.

Holmes, E., Li, J. V., Athanasiou, T., Ashrafian, H., & Nicholson, J. (2011). Understanding the role of gut microbiome–host metabolic signal disruption in health and disease. *Trends in Microbiology, 19*(7), 349–359.

Hooper, L. V., Midtvedt, T., & Gordon, J. I. (2002). How host-microbial interactions shape the nutrient environment of the mammalian intestine. *Annual Review of Nutrition, 22*(1), 283–307.

Hou, Q., Kwok, L. Y., Zheng, Y., Wang, L., Guo, Z., Zhang, J., et al. (2016). Differential fecal microbiota are retained in broiler chicken lines divergently selected for fatness traits. *Scientific Reports, 6*, 37376. https://doi.org/10.1038/srep37376

Hughes, R. J. (2008). Relationship between digesta transit time and apparent metabolisable energy value of wheat in chickens. *British Poultry Science, 49*(6), 716–720.

Ibarra, J. A., & Steele-Mortimer, O. (2009). *Salmonella*–the ultimate insider. *Salmonella* virulence factors that modulate intracellular survival. *Cellular Microbiology, 11*(11), 1579–1586.

Jansson, J., Willing, B., Lucio, M., Fekete, A., Dicksved, J., Halfvarson, J., et al. (2009). Metabolomics reveals metabolic biomarkers of Crohn's disease. *PLoS One, 4*(7), 6386.

Jenssen, H., Hamill, P., & Hancock, R. E. (2006). Peptide antimicrobial agents. *Clinical Microbiology Reviews, 19*(3), 491–511.

Józefiak, D., Rutkowski, A., & Martin, S. A. (2004). Carbohydrate fermentation in the avian ceca: A review. *Animal Feed Science and Technology, 113*(1), 1–15.

Kato, L. M., Kawamoto, S., Maruya, M., & Fagarasan, S. (2014). The role of the adaptive immune system in regulation of gut microbiota. *Immunodeficiency Reviews, 260*(1), 67–75.

Kawamoto, S., Maruya, M., Kato, L. M., Suda, W., Atarashi, K., Doi, Y., et al. (2014). Foxp3+ T cells regulate immunoglobulin a selection and facilitate diversification of bacterial species responsible for immune homeostasis. *Immunity, 41*(1), 152–165.

Keller, L. H., Benson, C. E., Krotec, K., & Eckroade, R. J. (1995). *Salmonella* Enteritidis colonization of the reproductive tract and forming and freshly laid eggs of chickens. *Infection and Immunity, 63*(7), 2443–2449.

Kim, D. K., Lillehoj, H. S., Lee, S. H., Jang, S. I., & Bravo, D. (2010). High-throughput gene expression analysis of intestinal intraepithelial lymphocytes after oral feeding of carvacrol, cinnamaldehyde, or *Capsicum oleoresin*. *Poultry Science, 89*(1), 68–81.

Koenen, M. E., Kramer, J., Van Der Hulst, R., Heres, L., Jeurissen, S. H. M., & Boersma, W. J. A. (2004). Immunomodulation by probiotic lactobacilli in layer-and meat-type chickens. *British Poultry Science, 45*(3), 355–366.

Kogut, M. H., & Arsenault, R. J. (2016). Gut health: The new paradigm in food animal production. *Frontiers in Veterinary Science, 3*, 71. https://doi.org/10.3389/fvets.2016.00071

Kollanoor-Johny, A., Mattson, T., Baskaran, S. A., Amalaradjou, M. A., Babapoor, S., March, B., et al. (2012). Reduction of *Salmonella enterica* serovar Enteritidis colonization in 20-day-old broiler chickens by the plant-derived compounds trans-cinnamaldehyde and eugenol. *Applied and Environmental Microbiology, 78*(8), 2981–2987.

Koutsos, E. A., & Arias, V. J. (2006). Intestinal ecology: Interactions among the gastrointestinal tract, nutrition, and the microflora. *Journal of Applied Poultry Research, 15*(1), 161–173.

Lai, Y., Di Nardo, A., Nakatsuji, T., Leichtle, A., Yang, Y., Cogen, A. L., et al. (2009). Commensal bacteria regulate toll-like receptor 3–dependent inflammation after skin injury. *Nature Medicine, 15*(12), 1377–1382.

Latshaw, J. D., & Zhao, L. (2011). Dietary protein effects on hen performance and nitrogen excretion. *Poultry Science, 90*(1), 99–106.

Le Roy, C. I., Mappley, L. J., La Ragione, R. M., Woodward, M. J., & Claus, S. P. (2016). NMR-based metabolic characterization of chicken tissues and biofluids: A model for avian research. *Metabolism, 12*(10), 157.

Lee, Y. K., & Mazmanian, S. K. (2010). Has the microbiota played a critical role in the evolution of the adaptive immune system? *Science, 330*(6012), 1768–1773.

Lenz, E. M., Bright, J., Knight, R., Wilson, I. D., & Major, H. (2004). A metabonomic investigation of the biochemical effects of mercuric chloride in the rat using 1H NMR and HPLC-TOF/MS: Time dependant changes in the urinary profile of endogenous metabolites as a result of nephrotoxicity. *Analyst, 129*(6), 535–541.

Ley, R. E., Bäckhed, F., Turnbaugh, P., Lozupone, C. A., Knight, R. D., & Gordon, J. I. (2005). Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences, 102*(31), 11070–11075.

Ley, R. E., Lozupone, C. A., Hamady, M., Knight, R., & Gordon, J. I. (2008). Worlds within worlds: Evolution of the vertebrate gut microbiota. *Nature Reviews. Microbiology, 6*(10), 776.

Li, X., Swaggerty, C. L., Kogut, M. H., Chiang, H. I., Wang, Y., Genovese, K. J., et al. (2010). Gene expression profiling of the local cecal response of genetic chicken lines that differ in their susceptibility to *Campylobacter jejuni* colonization. *PLoS One, 5*(7), 11827.

Lin, J. (2011). Effect of antibiotic growth promoters on intestinal microbiota in food animals: A novel model for studying the relationship between gut microbiota and human obesity? *Frontiers in Microbiology, 2*, 53. https://doi.org/10.3389/fmicb.2011.00053

Lu, J., Idris, U., Harmon, B., Hofacre, C., Maurer, J. J., & Lee, M. D. (2003). Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. *Applied and Environmental Microbiology, 69*, 6816–6824.

Lumpkins, B. S., Batal, A. B., & Lee, M. D. (2010). Evaluation of the bacterial community and intestinal development of different genetic lines of chickens. *Poultry Science, 89*(8), 1614–1621.

Luo, Y. H., Peng, H. W., Wright, A. D., Bai, S. P., Ding, X. M., Zeng, Q. F., et al. (2003). Broilers fed dietary vitamins harbor higher diversity of cecal bacteria and higher ratio of *Clostridium, Faecalibacterium*, and *Lactobacillus* than broilers with no dietary vitamins revealed by 16S rRNA gene clone libraries. *Poultry Science, 92*(9), 2358–2366.

Macpherson, A. J., & Harris, N. L. (2004). Opinion: Interactions between commensal intestinal bacteria and the immune system. *Nature Reviews Immunology, 4*(6), 478–485.

Maisonnier, S., Gomez, J., Bree, A., Berri, C., Baeza, E., & Carre, B. (2003). Effects of microflora status, dietary bile salts and guar gum on lipid digestibility, intestinal bile salts, and histomorphology in broiler chickens. *Poultry Science, 82*(5), 805–814.

Malik-Kale, P., Jolly, C. E., Lathrop, S., Winfree, S., Luterbach, C., & Steele-Mortimer, O. (2011). *Salmonella* - at home in the host cell. *Frontiers in Microbiology, 2*, 125. https://doi.org/10.3389/fmicb.2011.00125

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. *The American Journal of Clinical Nutrition, 79*(5), 727–747.

Marin, L., Miguélez, E. M., Villar, C. J., & Lombó, F. (2015). Bioavailability of dietary polyphenols and gut microbiota metabolism: Antimicrobial properties. BioMed Research International, 2015, 905215. https://doi.org/10.1155/2015/905215.

Markley, J. L., Brüschweiler, R., Edison, A. S., Eghbalnia, H. R., Powers, R., Raftery, D., et al. (2017). The future of NMR-based metabolomics. *Current Opinion in Biotechnology, 43*, 34–40.

Matulova, M., Rajova, J., Vlasatikova, L., Volf, J., Stepanova, H., Havlickova, H., et al. (2012). Characterization of chicken spleen transcriptome after infection with *Salmonella enterica* serovar Enteritidis. *PLoS One, 7*(10), 48101.

Mead, G. C. (1989). Microbes of the avian cecum: Types present and substrates utilized. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology, 252*(S3), 48–54.

Meade, K. G., Narciandi, F., Cahalane, S., Reiman, C., Allan, B., & O’Farrelly, C. (2009). Comparative in vivo infection models yield insights on early host immune response to *Campylobacter* in chickens. *Immunogenetics, 61*(2), 101–110.

MMWR, Centers for Disease Control and Prevention. (2013). Surveillance for foodborne disease outbreaks—United States, 2013. *Morbidity and Mortality Weekly Report, 62*, 1–34.

Morris, S. C. (2003). Life’s Solution: Inevitable Humans in a Lonely Universe. Cambridge, United Kingdom: Cambridge University Press.

Nicholson, J. K., Holmes, E., Kinross, J., Burcelin, R., Gibson, G., Jia, W., et al. (2012). Host-gut microbiota metabolic interactions. *Science, 336*(6086), 1262–1267.

Nicolaou, N., & Goodacre, R. (2008). Rapid and quantitative detection of the microbial spoilage in milk using Fourier transform infrared spectroscopy and chemometrics. *Analyst, 133*(10), 1424–1431.

O’Reilly, E. L., Burchmore, R. J., Sparks, N. H., & Eckersall, P. D. (2017). The effect of microbial challenge on the intestinal proteome of broiler chickens. *Protein Science, 15*(1), 10.

Oakley, B. B., Lillehoj, H. S., Kogut, M. H., Kim, W. K., Maurer, J. J., & Pedroso, A. (2014). The chicken gastrointestinal microbiome. *FEMS Microbiology Letters, 360*(2), 100–112.

Ozdal, T., Sela, D. A., Xiao, J., Boyacioglu, D., Chen, F., & Capanoglu, E. (2016). The reciprocal interactions between polyphenols and gut microbiota and effects on bioaccessibility. *Nutrition, 8*(2), 78.

Palm, N. W., de Zoete, M. R., & Flavell, R. A. (2015). Immune–microbiota interactions in health and disease. *Clinical Immunology, 159*(2), 122–127.

Pan, D., & Yu, Z. (2014). Intestinal microbiome of poultry and its interaction with host and diet. *Gut Microbes, 5*(1), 108–119.

Panda, A. K., Rao, S. R., Raju, M. V. L. N., & Sunder, G. S. (2009). Effect of butyric acid on performance, gastrointestinal tract health and carcass characteristics in broiler chickens. *Asian-Australasian Journal of Animal Sciences, 22*(7), 1026–1031.

Park, S. H., Lee, S. I., & Ricke, S. C. (2016). Microbial populations in naked neck chicken ceca raised on pasture flock fed with commercial yeast cell wall prebiotics via an illumina MiSeq platform. *PLoS One, 11*(3), 0151944.

Pawliszyn, J. (Ed.). (1997). *Solid phase microextraction: Theory and practice*. New York: Wiley.

Perumbakkam, S., Hunt, H. D., & Cheng, H. H. (2014). Marek’s disease virus influences the core gut microbiome of the chicken during the early and late phases of viral replication. *FEMS Microbiology Ecology, 90*(1), 300–312.

Qu, A., Brulc, J. M., Wilson, M. K., Law, B. F., Theoret, J. R., & Joens, L. A. (2008). Comparative metagenomics reveals host specific metavirulomes and horizontal gene transfer elements in the chicken cecum microbiome. *PLoS One, 3*(8), e2945.

Quirk, P. G., King, G. F., Campbell, I. D., & Boyd, C. A. (1989). Quantitation of metabolites of isolated chicken enterocytes using NMR spectroscopy. *American Journal of Physiology-Gastrointestinal and Liver Physiology, 256*(2), G423–G429.

Rehman, H. U., Vahjen, W., Awad, W. A., & Zentek, J. (2007). Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens. *Archives of Animal Nutrition, 61*(5), 19–335.

Ridaura, V. K., Faith, J. J., Rey, F. E., Cheng, J., Duncan, A. E., Kau, A. L., et al. (2013). Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science, 341*(6150), 1241214.

Ridlon, J. M., Kang, D. J., & Hylemon, P. B. (2006). Bile salt biotransformations by human intestinal bacteria. *Journal of Lipid Research, 47*(2), 241–259.

Rinttilä, T., & Apajalahti, J. (2013). Intestinal microbiota and metabolites—Implications for broiler chicken health and performance1. *Journal of Applied Poultry Research, 22*(3), 647–658.

Roberts, L. D., Souza, A. L., Gerszten, R. E., & Clish, C. B. (2012). Targeted metabolomics. *Current Protocols in Molecular Biology, 98*(30.2), 30.2.1–30.2.24.

Roessner, U., Wagner, C., Kopka, J., Trethewey, R. N., & Willmitzer, L. (2000). Simultaneous analysis of metabolites in potato tuber by gas chromatography–mass spectrometry. *The Plant Journal, 23*(1), 131–142.

Roto, S. M., Rubinelli, P. M., & Ricke, S. C. (2015). An introduction to the avian gut microbiota and the effects of yeast-based prebiotic-type compounds as potential feed additives. *Frontiers in Veterinary Science, 2*, 28. https://doi.org/10.3389/fvets.2015.00028

Saengkerdsub, S., Anderson, R. C., Wilkinson, H. H., Kim, W. K., Nisbet, D. J., & Ricke, S. C. (2007a). Identification and quantification of methanogenic archaea in adult chicken ceca. *Applied and Environmental Microbiology, 73*(1), 353–356.

Saengkerdsub, S., Herrera, P., Woodward, C. L., Anderson, R. C., Nisbet, D. J., & Ricke, S. C. (2007b). Detection of methane and quantification of methanogenic archaea in feces from young broiler chickens using real-time PCR. *Letters in Applied Microbiology, 45*(6), 629–634.

Scallan, E., Griffin, P. M., Angulo, F. J., Tauxe, R. V., & Hoekstra, R. M. (2011). Foodborne illness acquired in the United States—unspecified agents. *Emerging Infectious Diseases, 17*(1), 16.

Schabauer, L., Wenning, M., Huber, I., & Ehling-Schulz, M. (2014). Novel physico-chemical diagnostic tools for high throughput identification of bovine mastitis associated gram-positive, catalase-negative cocci. *BMC Veterinary Research, 10*(1), 156.

Scheppach, W. (1994). Effects of short chain fatty acids on gut morphology and function. *Gut, 35*, 35–38.

Scheppach, W., Pomare, E. W., Elia, M., & Cummings, J. H. (1991). The contribution of the large intestine to blood acetate in man. *Clinical Science, 80*(2), 177–182.

Schokker, D., Veninga, G., Vastenhouw, S. A., Bossers, A., Bree, F. M., Kaal-Lansbergen, L. M., et al. (2015). Early life microbial colonization of the gut and intestinal development differ between genetically divergent broiler lines. *BMC Genetics, 16*(1), 418.

Schroeder, B. O., & Bäckhed, F. (2016). Signals from the gut microbiota to distant organs in physiology and disease. *Nature Medicine, 22*(10), 1079–1089.

Sekirov, I., Russell, S. L., Antunes, L. C. M., & Finlay, B. B. (2010). Gut microbiota in health and disease. *Physiological Reviews, 90*(3), 859–904.

Sergeant, M. J., Constantinidou, C., Cogan, T. A., Bedford, M. R., Penn, C. W., & Pallen, M. J. (2014). Extensive microbial and functional diversity within the chicken cecal microbiome. *PLoS One, 9*(3), 91941.

Shai, Y. (1999). Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by α-helical antimicrobial and cell non-selective membrane-lytic peptides. *Biochimica et Biophysica Acta (BBA) - Biomembranes, 1462*(1), 55–70.

Shapaval, V., Schmitt, J., Møretrø, T., Suso, H. P., Skaar, I., Åsli, A. W., et al. (2013). Characterization of food spoilage fungi by FTIR spectroscopy. *Journal of Applied Microbiology, 114*(3), 788–796.

Shojadoost, B., Vince, A. R., & Prescott, J. F. (2012). The successful experimental induction of necrotic enteritis in chickens by *Clostridium perfringens*: A critical review. *Veterinary Research, 43*(1), 74.

Smirnov, K. S., Maier, T. V., Walker, A., Heinzmann, S. S., Forcisi, S., Martinez, I., et al. (2016). Challenges of metabolomics in human gut microbiota research. *International Journal of Medical Microbiology, 306*(5), 266–279.

Smith, E. A., & Macfarlane, G. T. (1998). Enumeration of amino acid fermenting bacteria in the human large intestine: Effects of pH and starch on peptide metabolism and dissimilation of amino acids. *FEMS Microbiology Ecology, 25*(4), 355–368.

Smolinska, A., Blanchet, L., Buydens, L. M., & Wijmenga, S. S. (2012). NMR and pattern recognition methods in metabolomics: From data acquisition to biomarker discovery: A review. *Analytica Chimica Acta, 750*, 82–97.

Stanley, D., Denman, S. E., Hughes, R. J., Geier, M. S., Crowley, T. M., Chen, H., et al. (2012). Intestinal microbiota associated with differential feed conversion efficiency in chickens. *Applied Microbiology and Biotechnology, 96*(5), 1361–1369.

Stanley, D., Geier, M. S., Hughes, R. J., Denman, S. E., & Moore, R. J. (2013). Highly variable microbiota development in the chicken gastrointestinal tract. *PLoS One, 8*(12), 84290.

Stanley, D., Hughes, R. J., & Moore, R. J. (2014). Microbiota of the chicken gastrointestinal tract: Influence on health, productivity and disease. *Applied Microbiology and Biotechnology, 98*(10), 4301–4310.

Struwe, W. B., Gough, R., Gallagher, M. E., Kenny, D. T., Carrington, S. D., Karlsson, N. G., et al. (2015). Identification of O-glycan structures from chicken intestinal mucins provides insight into *Campylobactor jejuni* pathogenicity. *Molecular and Cellular Probes, 14*(6), 1464–1477.

Swart, A. L., & Hensel, M. (2012). Interactions of *Salmonella enterica* with dendritic cells. *Virulence, 3*(7), 660–667. https://doi.org/10.4161/viru.22761

Taboada, E. N., Clark, C. G., Sproston, E. L., & Carrillo, C. D. (2013). Current methods for molecular typing of *Campylobacter* species. *Journal of Microbiological Methods, 95*(1), 24–31.

Tang, Y., Underwood, A., Gielbert, A., Woodward, M. J., & Petrovska, L. (2014). Metaproteomics analysis reveals the adaptation process for the chicken gut microbiota. *Applied and Environmental Microbiology, 80*(2), 478–485.

Taveirne, M. E., Theriot, C. M., Livny, J., & DiRita, V. J. (2013). The complete *Campylobacter jejuni* transcriptome during colonization of a natural host determined by RNAseq. *PLoS One, 8*(8), 73586.

Tellez, G., Higgins, S. E., Donoghue, A. M., & Hargis, B. M. (2006). Digestive physiology and the role of microorganisms. *Journal of Applied Poultry Research, 15*(1), 136–144.

Thibodeau, A., Fravalo, P., Yergeau, É., Arsenault, J., Lahaye, L., & Letellier, A. (2015). Chicken caecal microbiome modifications induced by *Campylobacter jejuni* colonization and by a non-antibiotic feed additive. *PLoS One, 10*(7), 0131978.

Topping, D. L., & Clifton, P. M. (2001). Short-chain fatty acids and human colonic function: Roles of resistant starch and nonstarch polysaccharides. *Physiological Reviews, 81*(3), 1031–1064.

Torok, V. A., Ophel-Keller, K., Loo, M., & Hughes, R. J. (2008). Application of methods for identifying broiler chicken gut bacterial species linked with increased energy metabolism. *Applied and Environmental Microbiology, 74*(3), 783–791.

Upadhyay, A., Arsi, K., Wagle, B. R., Shrestha, S., Upadhyaya, I., Bhargava, K., et al. (2017b). In-water supplementation of trans-cinnamaldehyde nanoemulsion reduces *Campylobacter jejuni* colonization in broiler chickens. *Poultry Science E-Supplement, 96*, 537.

Upadhyay, A., Upadhyaya, I., Arsi, K., Donoghue, A. M., & Donoghue, D. J. (2017a). Effect of in-water supplementation of trans-cinnamaldehyde nanoemulsion on *Campylobacter jejuni* colonization and cecal microbiome in broiler chickens. Annual Conference of the German Genetics Society 24.

Upadhyaya, I., Chen, C., Upadhyay, A., Yin, H., Nair, M. S., Khan, M. I., et al. (2015a). Effect of in-feed supplementation of *trans*-cinnamaldehyde and caprylic acid on chicken cecal microbiome in response to *Salmonella* Enteritidis. *Poultry Science E-Supplement, 94*(Suppl), 172.

Upadhyaya, I., Upadhyay, A., Arsi, K., Liyanage, R., Donoghue, A. M., Rath, N. C., et al. (2017). Plant-derived antimicrobial eugenol modulates *C. jejuni* proteome and virulence critical for colonization in chickens. *Poultry Science, 96*, 537.

Upadhyaya, I., Upadhyay, A., Kollanoor-Johny, A., Mooyottu, S., Baskaran, S. A., Yin, H., et al. (2015b). In-feed supplementation of trans-cinnamaldehyde reduces layer-chicken eggborne transmission of *Salmonella enterica* serovar Enteritidis. *Applied and Environmental Microbiology, 81*(9), 2985–2994.

Upadhyaya, I., Upadhyay, A., Yin, H., Nair, M. S., Bhattaram, V. K., Karumathil, D., et al. (2015c). Reducing colonization and eggborne transmission of *Salmonella* Enteritidis in layer chickens by in-feed supplementation of caprylic acid. *Foodborne Pathogens and Disease, 12*(7), 591–597.

USDA-ERS. (2012). *Cost estimates of foodborne illnesses*. Retrieved August 21, 2017, from https://www.ers.usda.gov/data-products/cost-estimates-of-foodborne-illnesses.aspx#48498

Vaidyanathan, S., Harrigan, G. G., & Goodacre, R. (Eds.). (2006). *Metabolome analyses: Strategies for systems biology*. New York: Springer.

van der Wielen, P. W., Biesterveld, S., Notermans, S., Hofstra, H., Urlings, B. A., & van Knapen, F. (2000). Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. *Applied and Environmental Microbiology, 66*(6), 2536–2540.

Van Duynhoven, J., Vaughan, E. E., Jacobs, D. M., Kemperman, R. A., Van Velzen, E. J., Gross, G., et al. (2011). Metabolic fate of polyphenols in the human superorganism. *Proceedings of the National Academy of Sciences, 108*, 4531–4538.

Van Immerseel, F., Cauwerts, K., Devriese, L. A., Haesebrouck, F., & Ducatelle, R. (2002). Feed additives to control *Salmonella* in poultry. *World's Poultry Science Journal, 58*(4), 501–513.

Verberkmoes, N. C., Russell, A. L., Shah, M., Godzik, A., Rosenquist, M., Halfvarsson, J., et al. (2008). *Shotgun metaproteomics of the human distal gut microbiota (No. LBNL-1134E)*. Berkeley, CA: Ernest Orlando Lawrence Berkeley National Laboratory.

Vernocchi, P., Del Chierico, F., & Putignani, L. (2016). Gut microbiota profiling: Metabolomics based approach to unravel compounds affecting human health. *Frontiers in Microbiology, 7*, 1144. https://doi.org/10.3389/fmicb.2016.01144

Vernocchi, P., Vannini, L., Gottardi, D., Del Chierico, F., Serrazanetti, D. I., Ndagijimana, M., et al. (2012). Integration of datasets from different analytical techniques to assess the impact of nutrition on human metabolome. *Frontiers in Cellular and Infection Microbiology, 2*, 156. https://doi.org/10.3389/fcimb.2012.00156

Videnska, P., Sisak, F., Havlickova, H., Faldynova, M., & Rychlik, I. (2013). Influence of *Salmonella enterica* serovar Enteritidis infection on the composition of chicken cecal microbiota. *BMC Veterinary Research, 9*(1), 140.

Waite, D. W., & Taylor, M. W. (2015). Exploring the avian gut microbiota: Current trends and future directions. *Frontiers in Microbiology, 6*, 673. https://doi.org/10.3389/fmicb.2015.00673

Walton, C., Fowler, D. P., Turner, C., Jia, W., Whitehead, R. N., Griffiths, L., et al. (2013). Analysis of volatile organic compounds of bacterial origin in chronic gastrointestinal diseases. *Inflammatory Bowel Diseases, 19*(10), 2069–2078.

Wei, S., Morrison, M., & Yu, Z. (2013). Bacterial census of poultry intestinal microbiome. *Poultry Science, 92*(3), 671–683.

Wong, J. M., De Souza, R., Kendall, C. W., Emam, A., & Jenkins, D. J. (2006). Colonic health: Fermentation and short chain fatty acids. *Journal of Clinical Gastroenterology, 40*(3), 235–243.

Woting, A., Pfeiffer, N., Loh, G., Klaus, S., & Blaut, M. (2014). Clostridium ramosum promotes high-fat diet-induced obesity in gnotobiotic mouse models. *MBio, 5*(5), 1530–1514.

Wu, S. B., Stanley, D., Rodgers, N., Swick, R. A., & Moore, R. J. (2014). Two necrotic enteritis predisposing factors, dietary fishmeal and *Eimeria* infection, induce large changes in the caecal microbiota of broiler chickens. *Veterinary Microbiology, 169*(3), 188–197.

Xin, H., Gates, R. S., Green, A. R., Mitloehner, F. M., Moore Jr., P. A., & Wathes, C. M. (2011). Environmental impacts and sustainability of egg production systems. *Poultry Science, 90*(1), 263–277.

Yang, D., Chertov, O., & Oppenheim, J. J. (2001). The role of mammalian antimicrobial peptides and proteins in awakening of innate host defenses and adaptive immunity. *Cellular and Molecular Life Sciences, 58*(7), 978–989.

Yeoman, C. J., Chia, N., Yildirim, S., Miller, M. E. B., Kent, A., Stumpf, R., et al. (2011). Towards an evolutionary model of animal-associated microbiomes. *Entropy, 13*(3), 570–594.

Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. *Nature, 415*(6870), 389–395.

Zheng, X., Xie, G., Zhao, A., Zhao, L., Yao, C., Chiu, N. H., et al. (2011). The footprints of gut microbial–mammalian co-metabolism. *Journal of Proteome Research, 10*(12), 5512–5522.

Zhou, D., & Galán, J. (2001). *Salmonella* entry into host cells: The work in concert of type III secreted effector proteins. *Microbes and Infection, 3*(14), 1293–1298.

Chapter 11
Molecular and New-Generation Techniques for Rapid Detection of Foodborne Pathogens and Characterization of Microbial Communities in Poultry Meat

Sang In Lee, Sun Ae Kim, Si Hong Park, and Steven C. Ricke

11.1 Introduction

Microbial contamination in poultry processing is an important component of the quality and safety associated with conversion of live birds to a final meat product that is ready for retail markets. In addition to the microbial contribution of the incoming birds from live-haul transportation, introduction of microorganisms via cross contamination can occur during all phases of poultry processing as well as during storage. The microbial communities associated with the poultry carcass is not only complex but can change as the carcass is processed and exposed to various environmental conditions such as treatment by antimicrobials and changes in temperature associated with the reduced temperature of the chiller tank (Ricke 2003; Oyarzabal 2005; Ricke et al. 2005; James et al. 2005; Sofos et al. 2013). While chilling is designed to reduce microbial load, some selection still occurs as microorganisms with more tolerance to cold temperatures are favored (James et al. 2005). Even though microbial populations are generally reduced after chilling, certain groups are favored that are associated with spoilage of the final product and thus can impact freshness and limit shelf life (Sofos et al. 2013). Likewise, certain microbial

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populations would be expected to be favored during storage and ultimately in retail markets.

Contamination and exposure to foodborne pathogens are also a concern as poultry is processed. The two primary pathogens generally identified with poultry include *Salmonella* and *Campylobacter jejuni* (Keener et al. 2004; Mead 2004; Oyarzabal 2005; Horrocks et al. 2009; Guerin et al. 2010; Cox et al. 2011; Handley et al. 2015; Rajan et al. 2017). Consequently, identification of these organisms is a critical part of providing a safe poultry meat product. Traditional culture-based microbial identification and enumeration methods have been the standard that the poultry industry and associated governmental regulatory agencies have relied on for assessing pathogen presence (Cox et al. 2011; Eberle and Kiess 2012; Gharst et al. 2013; Ricke et al. 2015). In general, such approaches have relied on some combination of enrichment and selection conditions present in the culture media to favor the growth of these organisms in the presence of a mixed microbial background. However, both organisms represent inherent challenges for conventional culturing and recovery from poultry that range from less selective than required or failure to recover injured cells (Eberle and Kiess 2012; Gorski 2012; Gharst et al. 2013; Oakley et al. 2011; Park et al. 2014). Consequently, interest has accelerated to focus more on the development of non-culture-based detection technologies that circumvent some of these problems. Although both immunological and molecular approaches have been explored as possible substitutes for detection and quantitation of certain foodborne pathogens, molecular techniques have received the most attention from a commercial development perspective (Maciorowski et al. 2006; Baker et al. 2016a, b).

A wide range of molecular techniques have been utilized in food microbiology and safety because rapid detection and typing analysis of foodborne pathogens can be directly related to public health. However, it is beginning to become apparent that general microbial ecologies of food, animals, and the processing environment are also important contributors. As consumer awareness of food safety and interest of functional food is increased, characterization of the microbiota that are present on meats and other food products along with an understanding of bacterial metabolism and the relationships with meat processing will become more important. Generating a detailed and comprehensive microbial profile of poultry meat products will require consistent, rapid, and accurate methodology to evaluate these aspects that are required for the poultry industry. Characterization of highly populated microbial regions on meat surfaces can be difficult. In the past decade, identifying and quantifying meat microbiota of food animal meat products had been traditionally limited to culture-based methods (Sofos et al. 2013). Molecular-based approaches such as polymerase chain reaction (PCR) including multiplex PCR and quantitative PCR, denaturing gradient gel electrophoresis (DGGE), and next-generation sequencing (NGS) methods are now coming into their own as detection and quantitation technologies for poultry products (Ricke et al. 2015). This review will not focus on specific organisms, but rather the focus will be on developments for particular molecular methods as well as current and potential pathogen and nonpathogen applications for poultry meat microbiology.

11.2 Multiplex PCR

When identifying pathogens in meat samples, Sutzko and Widmann (2017) identified several criteria for optimal pathogen detection including among others reproducibility (yields the same result in different laboratories) and repeatability (gives the same results in the same laboratory every time). These criteria would be considered relatively universal for evaluating any detection methodology whether culture or molecular based. Due to irreproducibility of various serotyping methods such as amplified fragment length polymorphism (AFLP) (Torpdahl and Ahrens 2004), random amplified polymorphic DNA (RAPD) polymerase chain reaction (PCR) (Hoorfar et al. 2000), and PCR-single-strand conformation polymorphism (SSCP) (Nair et al. 2002), multiplex PCR approaches were developed. Identifying different strains by multiplex PCR is considered easier compared to conventional PCR because the procedure is essentially identical as a conventional PCR but more rapid by hours due to its simultaneous amplification of multiple target regions. Multiplex PCR requires several primers that align with varying sizes of target genes and thus needs to be optimized so that all primer pairs can attach to the template strand at the same annealing temperature. This method was first introduced by Chamberlain et al. (1988) for detecting the majority of the deletions occurring in the Duchenne muscular dystrophy (DMD) gene. In addition, multiplex PCR has been utilized by Ballabio et al. (1990) to screen for the steroid sulfatase (STS) gene in patients with STS deficiency. Multiplex PCR had also been modified for genotyping microsatellite (SSR) and single-nucleotide polymorphism (SNP) by Hayden et al. (2008).

One of the advantages of multiplex PCR is that it provides internal controls (Edwards and Gibbs 1994) which means by amplifying multiple fragments, amplicons can act as internal controls for each other and thus can reveal false negatives. For example, the failure of fragment amplification can be determined if the noncontiguous deletions show no detectable bands because major gene deletions are usually contiguous (Chamberlain et al. 1992). Multiplex PCR can also be used as an indicator of template quality by determining several loci at the same time with greater sensitivity than Southern blot analysis. According to Chamberlain et al. (1992), multiplex PCR analysis revealed single exon deletion mutations which were not detected by Southern blot analysis, and 82% of those deletions detected by Southern blot analysis were also detected by multiplex PCR. By targeting multiple sequences, evaluation of amplification efficiency can be more accurate.

11.2.1 Microbial Pathogen Identification

One of the major applications of multiplex PCR is the identification of bacterial pathogens. Because multiplex PCR amplifies various genes simultaneously, specific bacterial species or strains can be differentiated. Numerous research and review articles have been published on the application of multiplex PCR approaches to

detect and identify bacterial pathogens in water, shellfish, animal feeds, respiratory tract, clinical and agricultural samples, and other sources (Bej et al. 1990; Brasher et al. 1998; Kong et al. 2002; Panicker et al. 2004; Maciorowski et al. 2005; Kim et al. 2007; Pacheco et al. 2007; Jarquin et al. 2009; Park et al. 2014; Baker et al. 2016a). Commonly targeted pathogens include *Campylobacter*, *E. coli* O157:H7, *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, and fungi. In addition, viral DNA can be amplified by multiplex PCR to screen tissue samples with infectious disease. Viruses including human papillomavirus (HPV), human immunodeficiency virus type 1 (HIV-1), human T-cell leukemia viruses, human T-lymphotrophic virus types I and II, hepatitis B virus, parvovirus, and hog viruses have been screened or detected by multiplex PCR assay in several studies (Sevall 1990; Vandenvelde et al. 1990; Sunzeri et al. 1991; Wattel et al. 1992; Repp et al. 1993; Wirz et al. 1993).

Salmonella and *E. coli* O157:H7 are typically classified as the foodborne pathogens of the greatest concern, and multiplex PCR technique enabled considerable reduction in detection time by days (Mahon et al. 1994; Park et al. 2014; Baker et al. 2016b). Baker et al. (2016a) also suggested that multiplex PCR can be used to distinguish closely related microbial species by developing genus- and species-specific primers via BLAST search targeting. In summarizing different cultural and molecular methods used for *Campylobacter*, Gharst et al. (2013) concluded that the multiplex PCR technique would be considered the most rapid means for identifying species of *Campylobacter*. Park and Ricke (2015) successfully developed a multiplex PCR assay that could simultaneously detect *Salmonella* genus, *Salmonella* subsp. I, *S*. Enteritidis, *S*. Heidelberg, and *S*. Typhimurium after spiking the corresponding isolates on chicken breast. Being able to detect *Salmonella* at the genus level is particularly useful as it allows for determination of whether a poultry sample is generally positive for *Salmonella* before conducting further classification.

11.2.2 Mutation Detection by DNA Typing

Differentiating genera and species of foodborne pathogens with conventional molecular typing tools can be done easily due to the distinct genomic differences. However, particularly with *Salmonella* this can become much more difficult when attempting to delineate among strains within the same serovar where genomic differences are nearly undetectable. Whole-genome sequencing has provided the means to differentiate genomes at the single-nucleotide level, and this has proven useful for tracking specific foodborne pathogen strains during foodborne disease outbreaks. Single-nucleotide polymorphism (SNP) refers to single-nucleotide variation in the genome when compared to otherwise similar genomes (Boxrud 2010; Ricke et al. 2015). Therefore, SNP sequence genotyping can become a critical unit for determining genetic variation. Conventional methods to detect SNP consist of two steps, amplification of target sequence and detection of the SNP (Ye et al. 1992, 2001). Since multiplex PCR can utilize several markers simultaneously, efficiency

and speed of SNP detection are improved. Cheng et al. (2004) employed multiplex PCR to rapidly detect mutations in *Mycobacterium tuberculosis*. Cheng et al. (2004) detected mutations in DNA fragments more rapidly within hours, compared to the parental single-stranded conformation polymorphism approach which requires more than 10 h (Cheng et al. 2004). Duchenne/Becker muscular dystrophy (DMD/BMD) is an example of human disease caused by a gene deletion in a specific region (Chamberlain et al. 1988). By targeting the steroid sulfatase gene with multiplex PCR, the respective deleted or altered gene could be identified (Chamberlain et al. 1988; Ballabio et al. 1990).

Another application of multiplex PCR is DNA typing to determine genetic linkage and mapping (Towbin et al. 1993; Neff et al. 1999). The DNA typing of an individual can be achieved more accurately by multiplex PCR targeting repetitive DNA polymorphisms because examining multiple loci decreases probability of identical alleles in two individuals. Amplifying short tandem repeats (STRs) in close proximity by multiplex PCR can be used to screen for disease linkages because STRs are highly polymorphic and numerous and may be co-amplified without overlapping size ranges (Beckmann and Weber 1992; Edwards et al. 1992).

11.3 Quantitative PCR (qPCR)

Quantitative PCR (qPCR) is also known as real-time PCR and refers to a method to determine absolute or relative amount of target sequence in a high-throughput format. Real-time PCR has often been confused with RT-PCR which is the abbreviation for reverse transcriptase PCR (Mackay 2007). The RT-PCR is a commonly used technique to detect RNA expression through creation of complementary DNA (cDNA) (Freeman et al. 1999). The qPCR approach is considered to be more sensitive, rapid, and safer compared to conventional PCR because a gel for amplicon confirmation is not needed and a radioactive reagent or chemical such as ethidium bromide is not involved (Arya et al. 2014). The qPCR commonly consists of three oligos, a pair of primers and a probe (Smith and Osborn 2009). Probes are designed to hybridize 100% to the amplified sequence and typically exhibit higher melting temperatures than the corresponding primers to allow annealing during the extension phase (Smith and Osborn 2009). When hybridized probes are cleaved by nuclease activity, fluorescence signals are released which are proportional to the target copy numbers (Smith and Osborn 2009). Two common methods of qPCR assay are utilization of SYBR green and TaqMan probes (Holland et al. 1991; Livak et al. 1995a; Wittwer et al. 1997; Houghton and Cockerill 2006). The SYBR green molecules release high fluorescent signals when they bind to double-stranded DNA, and weak signals will be detected if the molecules are not bound to the DNA strand (Wilhelm and Pingoud 2003).

One of the pitfalls of the SYBR green assay is that the dye can also bind to non-specific double-stranded DNA and thus generate false quantification values (Deprez et al. 2002). To overcome this problem, melting curve analysis is essential. According

to Wilhelm and Pingoud (2003) and Bustin (2002), primer dimers which are primer molecules that have hybridized to each other and non-specific double-stranded DNA can be distinguished by melting curve analysis (Bustin 2002; Wilhelm and Pingoud 2003; Smith and Osborn 2009). Taqman probes are considered as sequence-specific DNA probes because they are designed to hybridize to an amplified sequence (VanGuilder et al. 2008). The qPCR by Taqman probe is based on the use of the 5′ nuclease assay described by Holland et al. (1991) and dual-labeled fluorogenic hybridization probes (Holland et al. 1991; Lee et al. 1993; Bassler et al. 1995; Livak et al. 1995a, b). The Taqman probe consists of the reporter group at the 5′ end and the quencher group at the 3′ end that hybridizes during the extension phase. Reporter groups do not emit signals when the quencher group is present (Heid et al. 1996). However, once the quencher group is separated by nuclease activity, the reporter group emits a fluorescence signal (Heid et al. 1996). The qPCR can be applied for diagnostic and microbiological uses, gene quantification, and genotyping (Espy et al. 2006; Smith and Osborn 2009). The disadvantages of qPCR include the requirement for sequence data to the target gene of interest, binding of the dye to non-specific amplicons, incompatibility of the system with some fluorogenic chemistries, and inability to monitor amplicon size (Smith and Osborn 2009; Arya et al. 2014).

11.3.1 Clinical and Food Applications of qPCR

The introduction of qPCR methods in clinical microbiology has improved the detection of infectious disease agents and improved patient management and care (Sails 2013). For example, a wide range of viral diseases have been diagnosed by quantitative PCR (Niesters 2002). Also, qPCR has improved detection and quantification of numerous respiratory, gastrointestinal pathogens, enteric parasites, malaria parasites, leishmania organisms, and even viruses (Polley et al. 2011). Improved ability of qPCR is particularly useful when more slowly growing or poorly culturable bacteria need to be detected, for example, *Anaplasma phagocytophila*, *Bartonella henselae*, *Mycoplasma pneumoniae*, or *Chlamydophila* (Espy et al. 2006). The conventional PCR approach to these bacteria is difficult because some organisms elicit substances that inhibit PCR chemistry; thus sensitivity is greatly compromised (Espy et al. 2006). Increasing infection rates of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* species (VRE) in the US hospitals (Diekema et al. 2004) are considered tremendous threats to public health; thus the ability of qPCR to reduce detection time by several days compared to culture-based methods and simplified overall processes offers opportunities for more rapid appraisal (Niesters 2002).

In addition, qPCR can be utilized in fields as diverse as food safety, food spoilage, and fermentation and for the microbial risk assessment of water quality and in public health protection because molecular-based qPCR approaches are faster, more sensitive, and specific than previous standard approaches which are culture-based

methods (Postollec et al. 2011). Due to its advantages, the International Organization for Standardization (ISO) established the standard to detect foodborne pathogens utilizing PCR because of its ability to detect subdominant populations, dead or viable but non-cultivable cells (Postollec et al. 2011). In addition, dynamics and activities of genes can be studied by contrasting gene expression via combining qPCR with reverse transcript PCR (Postollec et al. 2011). Lee et al. (2007) were able to successfully demonstrate the power of reverse transcript PCR by analyzing genes associated with enterotoxin produced by *Staphylococcus aureus* suggesting reverse transcript PCR is very effective in evaluating gene expression.

11.3.2 Genotyping

The utilization of qPCR in clinical facilities has allowed for the quantification and characterization of virus strains such as the hepatitis B virus (Yeh et al. 2004). Yeh et al. (2004) reported an approach for one-step quantification of HBV genotypes A–G and genotyping of HBV genotypes B and C with one set of primers and probes that could overcome the difficulty of genotyping due to genetic diversity. Liu et al. (2003) successfully differentiated the number of gene copies in a mouse model using a qPCR assay. Liu et al. (2003) also emphasized that the qPCR assay is less labor-intensive than chromosome analysis (Davisson and Akeson 1987; Liu et al. 2003) while being more sensitive and reliable than other DNA-based methods such as Southern blot analysis. In addition, qPCR has allowed for the genotyping of mice at any age, whereas the chromosomal method was limited to mice over 6 weeks of age.

11.3.3 Foodborne Pathogen Quantification and Other Applications

Numerous studies have been conducted to quantify *Salmonella* from a wide range of food commodities including chicken carcass rinses, ground beef, ground pork, and raw milk (Chen et al. 1997). According to Hein et al. (2006), after enrichment the limit of detection to quantify *Salmonella* from chicken meat samples by qPCR was less than 5 CFU. In addition, Wang et al. (2007) demonstrated a rapid and simultaneous quantification method by combining qPCR and multiplex PCR and were able to successfully detect *E. coli* O157:H7, *Salmonella*, and *Shigella* from ground beef samples. According to Wang et al. (2007), the detection range of multiplex qPCR was 10^2 to 10^9, 10^3 to 10^9, and 10^1 to 10^8 CFU/mL for *E. coli*, *Salmonella*, and *Shigella*, respectively. In addition Elizaquível et al. (2013) reported a modified qPCR approach in order to detect only viable *Listeria monocytogenes*, *Salmonella*, and *E. coli* O157:H7 cells using propidium monoazide (PMA) (Elizaquível et al. 2013).

A qPCR approach was also used to detect genetically modified organisms (GMO). As GMOs are introduced to the market, scientists and consumers were concerned about its safety, and demand for analysis increased (Meyer 1999). According to Van den Bulcke (2010), the requirement for GMO screening methods are (1) appropriate approaches with adequate performance, scope, and legal basis, (2) suitable reference materials, and (3) decision support system which interprets the analytical results. The current approaches commonly used to identify specific single GMOs are the PCR-based technology (Holst-Jensen et al. 2003; Hernández et al. 2005). The GMOs are typically detected by species-specific PCR by reference targets, for example, the lectin gene is targeted for detection of roundup ready soybean (Berdal and Holst-Jensen 2001; Mafra et al. 2008). According to Brodmann et al. (2002), qPCR detection methods were developed for the four approved genetically modified maize variants.

More recently Kim et al. (2017a) combined a most probable number (MPN) titer plate assay with qPCR to increase the detection sensitivity for *S*. Typhimurium on chicken breast meat. The advantage of combining these two methodologies was the use of nonselective media in the MPN to shorten the incubation time for detectable quantities of *S*. Typhimurium to appear and the minimal volumes of media required for the MPN. The resulting MPN-qPCR-SIT (shortened incubation time) enabled quantitation requiring 7 h to complete quantification of *S*. Typhimurium. Further refinements of using richer media to ensure even more rapid growth and employing qPCR assays for other *Salmonella* serovars should expand the utility of this approach for practical applications. In addition, the nonselective media in the MPN provides an opportunity to use other PCR assays for other foodborne pathogens as well as nonpathogens such as key spoilage microorganisms.

11.4 Molecular Methodology for Characterizing the Meat Microbiome

11.4.1 Denaturing Gradient Gel Electrophoresis (DGGE)

Characterizing the microbiome is critical because such populations can be directly associated with gut health of the host and their performance (Noverr and Huffnagle 2004; Gill et al. 2006; Turnbaugh et al. 2006). A 16S rRNA amplification-based approach is a widely used technique for identification and classification of microorganisms because it is present in most microorganisms (Kuczynski et al. 2012). One of the initial techniques to fingerprint microbial ecology in environmental samples was electrophoretic separation via high-resolution polyacrylamide gels of low molecular weight rRNA molecules. Denaturing gradient gel electrophoresis (DGGE) was first introduced by Muyzer et al. (1993) to differentiate mixed microorganisms in a consortia mixture consisting of *Escherichia coli*, *Desulfovibrio desulfuricans*, *Microcoleus chthomoplastes*, *Desulfovibrio sapovorans*, and *Thiobacillus thioparus* from Leiden University and Wadden Sea sediment and Slufter sediment on the island of Texel, respectively. The PCR-based DGGE could be used to separate PCR amplicons of

uncharacterized microbial mixtures by their different melting points due to sequence variance of amplicons (Fischer and Lerman 1979; Myers et al. 1987), therefore allowing for a comparison of microbial communities (Hume et al. 2003; Oviedo-Rondón et al. 2006; Pedroso et al. 2006; Hanning and Ricke 2011). A key mechanism of this method was the attachment of a guanine and cytosine-rich region, the so-called GC clamp in the amplified fragments. Subsequent studies demonstrated that the presence of GC clamp increased the detection rate of sequence variance substantially (Myers et al. 1985; Sheffield et al. 1989). Because of this GC clamp, melted amplicons were able to halt migration once they reached the melting point threshold of the respective amplicons in the acrylamide gel. Substitution of the GC clamp, the ChemiClamp, attached a photoactivatable compound to the 5′ end of primer (Baker and Harayama 2004). However, limits of the ChemiClamp were clear. Initially the covalent bond PCR product could not be amplified correctly, and in addition, the possibility of UV light damaging PCR amplicons could not be resolved (Cariello et al. 1988). Also, the ChemiClamp could modify melting properties of PCR amplicons, making it hard to predict (Guldberg et al. 1998).

Limitations of DGGE include restricted fragment size, time required to complete a run, requirement of a well-trained technician, co-migration, and overestimation (Gafan and Spratt 2005). Fragment separation by DGGE and temperature gradient gel electrophoresis (TGGE) limits the size of the fragments up to 500 bp (Myers et al. 1985) which are also directly related to the amount of sequence information. In addition, Buchholz-Cleven et al. (1997) and Vallaeys et al. (1997) reported that if an organism exhibits certain levels of sequence variation, separation of DNA fragments was not possible (Buchholz-Cleven et al. 1997; Vallaeys et al. 1997).

Co-migration is also one of the pitfalls of DGGE and TGGE. For example, Nübel et al. (1996) reported that DGGE and TGGE resulted in the overestimation of the number of bacteria when some bacteria possessed multiple *rrN* operon sequences. Usage of degenerate primers in the PCR prior to DGGE can also result in an overestimation (Kowalchuk et al. 1997). Despite the development of NGS technologies which allow for a more directly quantitative statistical analysis of microbiome composition of the species level, DGGE can still be a viable technique due to complicated procedures, costs, and requirements of a well-trained technician to execute NGS. The advantage of DGGE is that it provides a simple, rapid visual profile of the microbial population of samples which can be very useful for pre-screening samples prior to NGS (Hanning and Ricke 2011). For example, a study by Yu et al. (2015) was conducted to study the microeukaryotic community from a sea sample by utilizing NGS and DGGE. Their study revealed that the NGS method using Illumina MiSeq revealed higher densities of the microeukaryotic community from samples compared to DGGE, yet no significant differences were detected in diversity of species.

11.4.1.1 Genetic Fingerprinting for Environmental Studies

One of the major utilities of DGGE is characterizing microbial ecology. Microbial fingerprinting by DGGE was used by Muyzer et al. (1993) when they amplified the gene fragments from widely distributed in bacteria and archaea using conventional

PCR (Saiki et al. 1988). Even though the molecular size of the amplicons was the same, amplicons were successfully separated by acrylamide gel because of the variant melting point properties resulting from the number of hydrogen bonds associated in guanine and cytosine. Besides analyzing only the patterns produced by DGGE, more detailed information could be obtained by using radioactively labeled oligonucleotide probes to sequence acrylamide gel fragments excised from DGGE (Amann et al. 1992; Muyzer and de Waal 1994). In addition, high-resolution melt (HRM) analysis profiling has been suggested as an additional approach post-DGGE analysis for the identification of DGGE bands (Porcellato et al. 2012). Teske et al. (1996) used DGGE to analyze distribution of sulfate-reducing bacterial population at three different time points during growth. Also Ferris et al. (1997) utilized PCR-DGGE to characterize the seasonal distribution of bacteria in hot springs and re-establishment of a microbial mat after removal of the entire cyanobacterial layer. For example, Dıez et al. (2001) successfully identified picoeukaryote diversity in natural marine assemblages. Also Dıez et al. (2001) compared the relative levels of specific microorganism rDNA using three different approaches including DGGE, T-RFLP, and gene cloning. Considering the technical differences, relative level values were reasonably comparable among the three techniques. In addition Dıez et al. (2001) noted that one of the pitfalls of DGGE is the variable quality of sequenced DGGE bands produced by Sanger sequencing.

11.4.1.2 Genetic Fingerprinting in Food Production Systems

Understanding and monitoring of bacterial communities in food matrices have been suggested as a mean to detect possible contamination by food safety-relevant organisms. Much of the early applications were associated with assessment of gastrointestinal microbial populations to changes in diets or other feed amendments (Wielen et al. 2002; Hume et al. 2003; Oviedo-Rondon et al. 2006; He et al. 2009; Park et al. 2013) and will not be discussed in the current review. The PCR-DGGE technique has also been utilized to study bacterial communities of various foods, for example, applications based on DGGE were first utilized to examine bacterial communities in pozol, a Mexican fermented maize dough (Ampe et al. 1999). Handschur et al. (2005) identified *Enterobacteriaceae* and *Pseudomonas libanensis* from processed salad samples. Porcellato et al. (2012) and Donner et al. (1996) reported the occurrence of species level of lactic acid bacteria in dairy products and observed changes in the enzymatic activity of cellulases by DGGE analysis. As DGGE exhibits its effectiveness in identifying bacterial community, the technique was widely utilized to fingerprint a variety of microorganism populations in various samples.

Since many food processing plants regulate the safety of their product by temperature, it is essential to monitor and determine the safety of the product. In a study by Handley et al. (2010), the microbial populations of poultry carcasses were examined by assessing banding patterns generated from DGGE, and they observed substantial transition over the time period of 44 to 50 h of post-chill process. Band pattern analysis by Handley et al. (2010) reported high similarities within sampled

groups. Also, the bands present from the beginning to the end of sampling time indicated which microbial groups were able to survive slaughter and evisceration process. Variation in detectable bands suggested that the environmental variation was occurring during processing; however, differences in bacterial levels and diversity may also be explained by other factors such as cross contamination from transport crates, equipment, or environmental conditions. In order to remove potential bias, Handley et al. (2010) noted that the processing procedures and equipment in the plants were identical.

Since meat slicers are widely used in ready-to-eat products including cheese, vegetables, and bread, it is important to keep the equipment safe from cross contamination of foodborne pathogens. Koo et al. (2013) utilized DGGE to analyze cross-contamination possibilities by slicer equipment used in deli meat retail processes. The DGGE technique revealed the similarities of overall populations of bacteria from the samples collected from slicer. Koo et al. (2013) successfully identified *Lactococcus lactis* and *Streptococcus thermophilus* from swabbed samples taken from the surfaces of the slicer. Overall microbial diversity of slicers analyzed by DGGE was similar between slicers, and the most densely populated part of the slicers was the blade guards.

11.4.1.3 Monitoring of Enrichment and Isolation of Bacteria

Originally, DGGE was developed to identify microbial communities and compare complexities among treatments; however, it also proved to be suitable to assess mixed populations of microorganisms (Muyzer and Smalla 1998). Santegoeds et al. (1996) and Ward et al. (1997) used DGGE to monitor enrichment cultures of bacteria in hot springs by 16S rRNA methods and successfully unveiled a diversity of bacterial populations in the enrichment cultures. By comparing bands produced by the respective 16S rRNA fragment, morphology, and the presence of biochemical markers, Garcia-Pichel et al. (1996) demonstrated that *Microcoleus chthonoplastes* represented a single, well-defined taxon with a ubiquitous distribution. In addition Teske et al. (1996) applied DGGE to analyze the constituents of a coculture by sequencing DGGE bands to design more selective conditions for isolation of *Desulfovibrio* and an *Arcobacter* isolate.

11.4.1.4 Gene Detection

Since DGGE approaches can be used to incorporate practical uses of melting points to differentiate variations in sequences, DGGE is also suitable for detecting microheterogeneity of genes. According to Nübel et al. (1996), TGGE band patterns produced by 16S rRNA fragments from pure cultures of *Paenibacillus polymyxa* exhibited distinct patterns. Different band patterns produced by pure cultures can serve as an indicator of sequence-based biodiversity and can be used to construct the corresponding phylogenetic tree. According to van der Luijt et al. (1997),

pathogenic mutations in the adenomatous polyposis cold (APC) gene which is responsible for familial adenomatous polyposis (FAP) could be identified by screening exons utilizing a DGGE technique combined with a protein truncation test and Southern blot analysis. van der Luijt et al. (1997) utilized DGGE for the small exon and used a protein truncation test for the large exon and successfully identified 65 pathogenic mutations from 105 Dutch FAP kindreds. Consequently, frameshifts and single-base substitution mutations could be identified.

11.4.1.5 Clone Library Screening

Colony hybridization and restriction fragment length polymorphism of cloned rRNA inserts (Moyer et al. 1996) has been one of the more highly utilized strategies to screen clone libraries. Both DGGE (Kowalchuk et al. 1997) and TGGE (Felske et al. 1998) analyses were also used to measure and estimate redundancy of cloned 16S rDNA inserts in the environment. Both DGGE and TGGE were employed to analyze PCR products which are amplified after 16S rRNA genes have been cloned in suitable vectors. By analyzing cloned inserts obtained from the environment, an indication of the representative members in the natural microbial community can be acquired. According to Muyzer and Smalla (1998), re-amplifying inserts with nested PCR will cluster clones together in groups, and each representative clone can be sequenced. Furthermore, DGGE analysis of PCR products from cloned inserts may give an indication of representative members in mixed culture samples. Kowalchuk et al. (1997) utilized DGGE to analyze PCR-amplified 16S rDNA fragments and successfully detected potential beta-subdivision ammonia oxidizers present in the dune samples.

11.4.1.6 Determining PCR and Cloning Biases

Keohavong and Thilly (1989) applied DGGE to determine the error rate of different DNA polymerases during DNA synthesis. They reported that DGGE permitted direct enumeration and identification of a point mutation caused by T4, modified T7, a Klenow fragment of polymerase I, and *Thermus aquaticus* (*Taq*) during PCR. Fidelity comparison of DNA amplification in the study was suitable because the base pairs were small and mutations could be detected by different concentrations of denaturant required for each amplicon. According to Keohavong and Thilly (1989), the most predominant mutations were transitions of G and C to A and T or vice versa with error rates varying from 3×10^{-6} to 2.1×10^{-4}. Keohavong and Thilly (1989) emphasized that reaction conditions such as temperature, dNTP, and concentration of salt may have an impact on mutations and error rate; however, error rates and mutations that predominantly existed were highly similar in four different templates indicating that the fidelity of the enzyme essentially remains constant during DNA synthesis. In the study by Eckert and Kunkel (1991), fidelity of various polymerases was compared using three techniques, DGGE, cloning, and M13mp2 in

an in vitro assay. The measured fidelities of PCR with *Taq* polymerases by DGGE were similar with fidelity measured by cloning PCR product and M13mp2 in an in vitro assay (Eckert and Kunkel 1991).

11.4.2 Next-Generation Sequencing (NGS)

Nucleic acid sequencing refers to a method for determining the order of nucleotides in DNA or RNA molecules (Sanger et al. 1973). However, first-generation sequencing or Sanger sequencing had obvious limits of time required and poor quality of beginning sequences. This resulted in demands for more economical and rapid methods in research and clinical labs which led to development of NGS approaches. The NGS provided a more high-throughput approach for the sequencing of millions of DNA fragments from a sample. Next-generation sequencing also became known as parallel sequencing because NGS can perform millions of sequencing reactions in parallel by micro-reactors and/or solid surfaces or beads (Reis-Filho 2009; Metzker 2010; Kwon and Ricke 2011). One of the notable differences between Sanger sequencing and NGS was the length of reads. While Sanger sequencing generated long reads (nearly a thousand base pairs (bp)), NGS generates millions of shorter reads (hundreds of bp) which can be quantified (John and Grody 2008; Morozova and Marra 2008; Fullwood et al. 2009; Stratton et al. 2009; Tucker et al. 2009; Voelkerding et al. 2009).

In the past decade, various NGS platforms have been developed including the Ion Torrent system of Life Technologies (Carlsbad, CA), MiSeq of Illumina (San Diego, CA), 454 pyrosequencing of Roche Diagnostics (Risch-Rotkreuz, Switzerland), and SOLiD of Applied Biosystems (Foster City, CA). Ion torrent by Life Technology utilizes a hydrogen ion-sensitive transistor, and its throughput is approximately 320 Mb per one run which runs for approximately 8 h. The Illumina Miseq needs to be run for 24 h and produces read lengths of 150 bp, and its throughput is from 1.0 to 1.4 Gb. Conversely, Roche 454 pyrosequencing technology runs for 10 hours and is able to produce read lengths of 400 bp and a throughput of 400 Mb per run. SoLiD requires over a week of run time and produces 15 Gb per day with a read length of 60 bp.

Next-generation sequencing can be applied to clinical diagnostics and therapeutics to determine the genetic cause of a disease by sequencing the protein-coding region of a respective gene (Saunders et al. 2012). Although whole-genome sequencing (WGS) is possible, sequencing hot spots for disease-causing mutations can be also effective because it is more rapid and more cost-effective. Targeting specific genomic regions can be accomplished by coupling NGS with DNA capturing methods (Ng et al. 2009).

Besides sequencing DNA, RNAseq also known as whole transcriptome shotgun sequencing (Morin et al. 2008) has been developed and can be applied to the study of gene expression, RNA sequencing, paired-end RNA sequencing, and small and noncoding RNA sequencing (Reis-Filho 2009; Wang et al. 2009). In addition,

RNAseq has allowed for the determination of exon and intron boundaries and the capability of observing cellular pathway alteration during infection (Qian et al. 2013). Eventually, RNAseq has led to more in-depth understanding of RNA editing events when combined with massive DNA sequencing. In addition, several modifications of NGS allowed assessment of DNA methylation and acetylation (Meissner et al. 2008; Lister and Ecker 2009) along with immune-chromatin microarray assays (Visel et al. 2009).

11.4.2.1 Whole-Exome Sequencing

The term exome refers to the sequences which are transcribed to RNA after introns are removed. Therefore, exome sequencing provides information on the protein-coding region of genome. Exon refers to the part of a gene that remains within the mature RNA after introns are removed. Human exons constitute approximately 1% of the total genome (Ng et al. 2009), and sequencing exons are known to be an efficient strategy to determine the genetic basis for gene disorders (Bamshad et al. 2011). Exome sequencing can also provide information of disease-causing mutations in pathogen. Lai-Cheong and McGrath (2011) successfully identified genes relevant to inherited skin disorders by exome sequencing. Disease-causing mutations in multiple genes have been identified by Cullinane et al. (2011) in a patient with oculocutaneous albinism and cogenital neutropena. The study by Cullinane et al. (2011) utilized whole-exome sequencing to align DNA sequence fragment of the patient to the corresponding reference genome to identify variation and detected 62,235 variations.

11.4.2.2 Targeted Sequencing

Sequencing of a specific region is preferred when the suspected disease is identified and the region of interest is already known. Compared to whole-exome sequencing, targeted sequencing is much more affordable, yields much higher coverage of genomic regions of interest, and reduces sequencing cost and time (Xuan et al. 2013). Also, cancer-type-specific treatments can be evaluated and aided by NGS targeting specific genomic region (Rehm 2013). Rehm (2013) reported that utilization of NGS is gradually extending its ability into clinical laboratories especially in diagnostics for hereditary disorders and prognostics of somatic cancers.

11.4.2.3 Microbial Community Analysis

High-throughput sequencing technology such as NGS, parallel sequencing, has revolutionized the study of microbial community analysis because NGS approaches can produce extensive and detailed data on bacterial composition which could be considered significant to both the health of host and in fermented food. Prokaryotic

16S rRNA and fungal ITS genes are typically targeted in the molecular surveillance of microbial communities. Sequencing millions of reads in a single run by NGS provides an incomparable amount of data with high-resolution optics at the molecular level. Hence, high computational power and storage became mandatory. Also, the gigabyte-sized data produced by the NGS platform requires programs to process NGS raw data to downstream analysis such as characterization of short amplicons, filtration/demultiplexing, operational taxonomic unit (OTU) selection, taxonomic assignment, and sequence alignment (Bokulich and Mills 2012).

Three open-source programs are available for processing data, quantitative insights into microbial ecology (QIIME) (Caporaso et al. 2010), Mothur (Schloss et al. 2009), and MG-RAST (Meyer et al. 2008). QIIME, Mothur, and MG-RAST open-source pipelines are capable of analyzing data by trimming, screening, and aligning sequences produced from community samples and furthermore able to calculate distance and OTUs between community sequence sample. According to Plummer et al. (2015), there were no significant differences detected at the phylum level, while genus levels exhibited differences when fecal samples of infants were analyzed targeting the 16S rRNA gene. Across the three pipelines, a total of 90 distinct genera were identified, and MG-RAST and Mothur shared the least genera at 39, while QIIME and Mothur exhibited higher similarity of 53 genera. Plummer et al. (2015) highlighted that the QIIME and Mothur pipelines have more powerful statistical capabilities than MG-RAST; however, Mothur excels the most in terms of flexibility. However Plummer et al. (2015) concluded that QIIME was more user friendly, required less time than Mothur, and was preferred for analyzing large datasets. Approximate analysis time for specific dataset used by Plummer et al. (2015) were 1 h, 10 h, and 2 days for QIIME, Mothur, and MG-RAST, respectively. The advantage of using MG-RAST pipeline is its accessibility to the public database and shotgun metagenomic datasets. Also, MG-RAST generates multi-fasta file for each sample, allowing the researcher to select particular reads for further analysis.

While microbiome examination of gastrointestinal tract microbial communities in live birds has been reported in numerous studies, only a few studies have used 16S-rRNA gene sequencing to characterize poultry processing and poultry meat microbial populations (Oakley et al. 2013; Rothrock et al. 2016; Kim et al. 2017b). In the most recent study, Kim et al. (2017b) used 16S-rRNA gene sequencing in a poultry pilot processing plant to determine chicken carcasses rinse microbiota responses during processing after exposure to commercial antimicrobials. They also compared microbial communities recovered from original chicken carcass rinsates versus colonies pooled from aerobic plate media used to enumerate bacteria from these same rinsates. Microbiome sequencing revealed *Firmicutes* to be the dominant phyla representing over half of the microbial composition in all processing steps compared to the decline in *Proteobacteria* over these same steps. Phyla recovered from the carcass rinsate, and the corresponding aerobic plate microbial populations were generally similar, but genera differed indicating that the plating media may have selectively influenced the recovery of certain groups of microorganisms present in the carcass rinsate microbial populations. These studies indicate microbiome mapping could be a useful tool for obtaining greater resolution on the impact

of external factors on the final composition of the poultry carcass microbiome as it enters the final stages of processing prior to storage. Such approaches may also offer opportunities to identify specific bacteria that might serve as ideal indicator organisms for assessing the efficacy of antimicrobials against potential foodborne pathogens present on the carcass. Indicator microorganisms are important as pathogens may not necessarily be uniformly distributed among carcasses and could also be at very low levels compared to the overall microbial population associated with the carcass (Handley et al. 2015). However, identifying candidate indicator organisms that are representative of pathogens and are also consistently present on the carcass during all stages of processing to provide baseline data remains a challenge. Microbiome sequencing offers a potential means to identify such organisms and develop specific quantitation assays for them. However, considerably more microbiome data will need to be generated to provide a large enough data base to establish the relationship between candidate indicator microorganisms and the corresponding foodborne pathogen.

11.5 Future Directions

It is anticipated that improvements in molecular techniques which can be applied to poultry meat will continue to be developed. For example, conventional PCR-agarose gel visualization is a useful technique; however, specificity and sensitivity of conventional PCR can be compromised by the possibility of a false-positive product (Sails 2013). The development of enzyme and probe hybridization molecules for detecting amplified product has led to accurate quantitation of target genes and the generation of real-time instrumentation and chemistry for PCR. The quantitative PCR alone provides data equivalent to the combination of conventional PCR and Southern blot analysis which are highly sensitive and specific. Because multiplex PCR requires several primer pairs with various properties such as specificity and optimal annealing temperature, optimization of the multiplex PCR can be difficult. However, once the assay has been optimized, the procedure of multiplexing becomes quite simple and provides more detailed information of template DNA because multiplex PCR can target species or strain specific fragments of DNA. Consequently, more rapid and accurate detection and characterization of pathogens in food safety or industry have become possible compared to previous enrichment culture-based methods which historically were the gold standard of detection. In addition, it can be applied to diagnose genetic and infectious disease accurately by screening for multiple loci simultaneously. The multiplex PCR assay can be also used to examine the relationship of genetic linkage between two or more sequences, environmental association, and host-parasite and disease-infection (Edwards and Gibbs 1994). This will also have utility for tracking pathogens during poultry processing and delineating points of origin as well as cross contamination.

Next-generation sequencing approaches developed following the wide-scale application of Sanger sequencing. These are also referred to as parallel sequencing methods because of their mechanisms involving sequencing millions of fragments simultaneously. The NGS can be applied to unlimited fields of studies involving DNA- or RNA-based technology. In addition, NGS approaches can not only be applied to sequence genome for genetic disorders but can also be applied to microbiome studies by targeting 16S rRNA; while the flexibility of NGS is useful, it can be inefficient, and the cost to implement NGS has historically been very expensive. Andersen et al. (2014) developed a methodology of enhancing NGS by adopting barcodes to PCR products prior to NGS library construction. The bar-coded library approach of NGS increased efficiency and decreased cost of library preparation by two thirds per sample. In addition to development of NGS, also called second-generation sequencing, development of third-generation sequencing and fourth-generation sequencing platforms is emerging (Schadt et al. 2010). The significance of third-generation sequencing is that amplification of template strands is no longer required which means availability of single-molecule real-time sequencing. The absence of amplification steps reduces the error that might occur during amplification (Ku and Roukos 2013). Third-generation sequencing technology includes the PacBio RS of Pacific BioSciences and the HeliScope sequencer of Helicos BioSciences. Fourth-generation sequencing technology is known as nanopore-based technology and is highlighted by the cost-effectiveness. Feng et al. (2015) introduced this technology to improve the potential of sequencing an entire human genome for less than $1000 or even less than $100.

11.6 Conclusions

The introduction of molecular technique advances into microbial characterization of poultry meat microbial populations has resulted in opportunities for a new level of comprehensive analyses of microbial ecology and pathogen presence during poultry processing. Molecular techniques such as DGGE, multiplex PCR, qPCR, and NGS can be utilized for the investigation of microbial composition and shift responses to environmental change which require more detailed investigations due to some of the inconsistent results occurring among studies. Since NGS methods are becoming more accessible, microbiome analysis of poultry meat products and poultry processing plant environments offer possibilities to develop extensive data profiles on microbial communities associated with poultry processing and how this may impact spoilage and shelf life. Microbiome mapping of poultry processing plants may provide new insights to sources of cross contamination as well as the impact of plant location and farm sources of incoming flocks on signature microbial communities within the plant environment. This level of resolution may in turn influence sanitation strategies to become more specific to a particular plant to maximize effectiveness against its specific resident microbial community.

References

Amann, R., Stromley, J., Devereux, R., Key, R., & Stahl, D. (1992). Molecular and microscopic identification of sulfate-reducing bacteria in multispecies biofilms. *Applied and Environmental Microbiology, 58*, 614–623.

Ampe, F., ben Omar, N., Moizan, C., Wacher, C., & Guyot, J.-P. (1999). Polyphasic study of the spatial distribution of microorganisms in Mexican pozol, a fermented maize dough, demonstrates the need for cultivation-independent methods to investigate traditional fermentations. *Applied and Environmental Microbiology, 65*, 5464–5473.

Andersen, J. D., Pereira, V., Pietroni, C., Mikkelsen, M., Johansen, P., Børsting, C., et al. (2014). Next-generation sequencing of multiple individuals per barcoded library by deconvolution of sequenced amplicons using endonuclease fragment analysis. *BioTechniques, 57*, 91–94.

Arya, M., Shergill, I. S., Williamson, M., Gommersall, L., Arya, N., & Patel, H. R. (2014). Basic principles of real-time quantitative PCR. *Expert Review of Molecular Diagnostics, 5*, 209–219.

Baker, C. A., Rubinelli, P. M., Park, S. H., Carbonero, F., & Ricke, S. C. (2016a). A Shiga toxin-producing *Escherichia coli* in food: Incidence, ecology, and detection strategies. *Food Control, 59*, 407–419.

Baker, C. A., Rubinelli, P. M., Park, S. H., & Ricke, S. C. (2016b). Immuno-based detection of Shiga toxin-producing pathogenic *Escherichia coli* in food—A review on current approaches and potential strategies for optimization. *Critical Reviews in Microbiology, 42*, 656–675.

Baker, P. W., & Harayama, S. (2004). An analysis of microorganisms in environments using denaturing gradient gel electrophoresis. *Environmental Microbiology: Methods and Protocols, 16*, 323–338.

Ballabio, A., Ranier, J. E., Chamberlain, J. S., Zollo, M., & Caskey, C. T. (1990). Screening for steroid sulfatase (STS) gene deletions by multiplex DNA amplification. *Human Genetics, 84*, 571–573.

Bamshad, M. J., Ng, S. B., Bigham, A. W., Tabor, H. K., Emond, M. J., Nickerson, D. A., et al. (2011). Exome sequencing as a tool for Mendelian disease gene discovery. *Nature Reviews Genetics, 12*, 745–755.

Bassler, H. A., Flood, S. J., Livak, K. J., Marmaro, J., Knorr, R., & Batt, C. A. (1995). Use of a fluorogenic probe in a PCR-based assay for the detection of *Listeria monocytogenes*. *Applied and Environmental Microbiology, 61*, 3724–3728.

Beckmann, J. S., & Weber, J. L. (1992). Survey of human and rat microsatellites. *Genomics, 12*, 627–631.

Bej, A. K., Mahbubani, M. H., Miller, R., DiCesare, J. L., Haff, L., & Atlas, R. M. (1990). Multiplex PCR amplification and immobilized capture probes for detection of bacterial pathogens and indicators in water. *Molecular and Cellular Probes, 4*, 353–365.

Berdal, K. G., & Holst-Jensen, A. (2001). Roundup Ready® soybean event-specific real-time quantitative PCR assay and estimation of the practical detection and quantification limits in GMO analyses. *European Food Research and Technology, 213*, 432–438.

Bokulich, N. A., & Mills, D. A. (2012). Next-generation approaches to the microbial ecology of food fermentations. *BMB Reports, 45*, 377–389.

Boxrud, D. (2010). Advances in subtyping of methods of disease pathogens. *Current Opinion in Biotechnology, 21*, 137–141.

Brasher, C. W., DePaola, A., Jones, D. D., & Bej, A. K. (1998). Detection of microbial pathogens in shellfish with multiplex PCR. *Current Microbiology, 37*, 101–107.

Brodmann, P. D., Ilg, E. C., Berthoud, H., & Herrmann, A. (2002). Real-time quantitative polymerase chain reaction methods for four genetically modified maize varieties and maize DNA content in food. *Journal of AOAC International, 85*, 646–653.

Buchholz-Cleven, B. E., Rattunde, B., & Straub, K. L. (1997). Screening for genetic diversity of isolates of anaerobic Fe (II)-oxidizing bacteria using DGGE and whole-cell hybridization. *Systematic and Applied Microbiology, 20*, 301–309.

Bustin, S. (2002). Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): Trends and problems. *Journal of Molecular Endocrinology, 29*, 23–39.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., et al. (2010). QIIME allows analysis of high-throughput community sequencing data. *Nature Methods, 7*, 335–336.

Cariello, N. F., Keohavong, P., Sanderson, B., & Thilly, W. G. (1988). DNA damage produced by ethidium bromide staining and exposure to ultraviolet light. *Nucleic Acids Research, 16*, 4157.

Chamberlain, J., Chamberlain, J., Fenwick, R., Ward, P., Caskey, C., Dimnik, L., et al. (1992). Diagnosis of Duchenne and Becker muscular dystrophies by polymerase chain reaction. A Multicenter Study. *JAMA, 267*, 2609–2615.

Chamberlain, J. S., Gibbs, R. A., Rainer, J. E., Nguyen, P. N., & Thomas, C. (1988). Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. *Nucleic Acids Research, 16*, 11141–11156.

Chen, S., Yee, A., Griffiths, M., Larkin, C., Yamashiro, C. T., Behari, R., et al. (1997). The evaluation of a fluorogenic polymerase chain reaction assay for the detection of *Salmonella* species in food commodities. *International Journal of Food Microbiology, 35*, 239–250.

Cheng, A. F., Yew, W. W., Chan, E. W., Chin, M. L., Hui, M. M., & Chan, R. C. (2004). Multiplex PCR amplimer conformation analysis for rapid detection of gyrA mutations in fluoroquinolone-resistant *Mycobacterium tuberculosis* clinical isolates. *Antimicrobial Agents and Chemotherapy, 48*, 596–601.

Cox, N. A., Cason, J. A., & Richardson, L. J. (2011). Minimization of *Salmonella* contamination on raw poultry. *Annual Review of Food Science and Technology, 2*, 75–95.

Cullinane, A. R., Vilboux, T., O'Brien, K., Curry, J. A., Maynard, D. M., Carlson-Donohoe, H., et al. (2011). Homozygosity mapping and whole-exome sequencing to detect SLC45A2 and G6PC3 mutations in a single patient with oculocutaneous albinism and neutropenia. *The Journal of Investigative Dermatology, 131*, 2017–2025.

Davisson, M., & Akeson, E. (1987). An improved method for preparing G-banded chromosomes from mouse peripheral blood. *Cytogenetic and Genome Research, 45*, 70–74.

Deprez, R. H. L., Fijnvandraat, A. C., Ruijter, J. M., & Moorman, A. F. (2002). Sensitivity and accuracy of quantitative real-time polymerase chain reaction using SYBR green I depends on cDNA synthesis conditions. *Analytical Biochemistry, 307*, 63–69.

Diekema, D. J., BootsMiller, B. J., Vaughn, T. E., Woolson, R. F., Yankey, J. W., Ernst, E. J., et al. (2004). Antimicrobial resistance trends and outbreak frequency in United States hospitals. *Clinical Infectious Diseases, 38*, 78–85.

Dıez, B., Pedrós-Alió, C., Marsh, T. L., & Massana, R. (2001). Application of denaturing gradient gel electrophoresis (DGGE) to study the diversity of marine picoeukaryotic assemblages and comparison of DGGE with other molecular techniques. *Applied and Environmental Microbiology, 67*, 2942–2951.

Donner, G., Schwarz, K., Hoppe, H., & Muyzer, G. (1996). Profiling the succession of bacterial populations in pelagic chemoclines. *Ergebnisse der Limnologie, 48*, 7–14.

Eberle, K. N., & Kiess, A. S. (2012). Phenotypic and genotypic methods for typing *Campylobacter jejuni* and *Campylobacter coli* in poultry. *Poultry Science, 91*, 255–264.

Eckert, K. A., & Kunkel, T. A. (1991). DNA polymerase fidelity and the polymerase chain reaction. *Genome Research, 1*, 17–24.

Edwards, A., Hammond, H. A., Jin, L., Caskey, C. T., & Chakraborty, R. (1992). Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. *Genomics, 12*, 241–253.

Edwards, M. C., & Gibbs, R. A. (1994). Multiplex PCR: Advantages, development, and applications. *Genome Research, 3*, S65–S75.

Elizaquível, P., Azizkhani, M., Sánchez, G., & Aznar, R. (2013). Evaluation of Zataria multiflora Boiss. essential oil activity against *Escherichia coli* O157: H7, *Salmonella enterica* and *Listeria monocytogenes* by propidium monoazide quantitative PCR in vegetables. *Food Control, 34*, 770–776.

Espy, M., Uhl, J., Sloan, L., Buckwalter, S., Jones, M., Vetter, E., et al. (2006). Real-time PCR in clinical microbiology: Applications for routine laboratory testing. *Clinical Microbiology Reviews, 19*, 165–256.

Felske, A., Wolterink, A., Van Lis, R., & Akkermans, A. D. (1998). Phylogeny of the main bacterial 16S rRNA sequences in Drentse A grassland soils (The Netherlands). *Applied and Environmental Microbiology, 64*, 871–879.

Feng, Y., Zhang, Y., Ying, C., Wang, D., & Du, C. (2015). Nanopore-based fourth-generation DNA sequencing technology. *Genomics, Proteomics & Bioinformatics, 13*, 4–16.

Ferris, M., Nold, S., Revsbech, N., & Ward, D. (1997). Population structure and physiological changes within a hot spring microbial mat community following disturbance. *Applied and Environmental Microbiology, 63*, 1367–1374.

Fischer, S. G., & Lerman, L. S. (1979). Length-independent separation of DNA restriction fragments in two-dimensional gel electrophoresis. *Cell, 16*, 191–200.

Freeman, W. M., Walker, S. J., & Vrana, K. E. (1999). Quantitative RT-PCR: Pitfalls and potential. *BioTechniques, 26*, 112–125.

Fullwood, M. J., Wei, C.-L., Liu, E. T., & Ruan, Y. (2009). Next-generation DNA sequencing of paired-end tags (PET) for transcriptome and genome analyses. *Genome Research, 19*, 521–532.

Gafan, G. P., & Spratt, D. A. (2005). Denaturing gradient gel electrophoresis gel expansion (DGGEGE)—an attempt to resolve the limitations of co-migration in the DGGE of complex polymicrobial communities. *FEMS Microbiology Letters, 253*, 303–307.

Garcia-Pichel, F., Prufert-Bebout, L., & Muyzer, G. (1996). Phenotypic and phylogenetic analyses show *Microcoleus chthonoplastes* to be a cosmopolitan cyanobacterium. *Applied and Environmental Microbiology, 62*, 3284–3291.

Gharst, G., Oyarzabal, O., & Hussain, S. (2013). Review of current methodologies to isolate and identify *Campylobacter* spp. from foods. *Journal of Microbiological Methods, 95*, 84–92.

Gill, S. R., Pop, M., DeBoy, R. T., Eckburg, P. B., Turnbaugh, P. J., Samuel, B. S., et al. (2006). Metagenomic analysis of the human distal gut microbiome. *Science, 312*, 1355–1359.

Gorski, L. (2012). Selective enrichment media bias the types of *Salmonella enterica* strains isolated from mixed strain cultures and complex enrichment broths. *PLoS One, 7*(4), e34722. https://doi.org/10.1371/journalpone.0034722

Guerin, M. T., Sir, C., Sargeant, J. M., Waddell, L., O'Connor, A. M., Wills, R. W., et al. (2010). The change in prevalence of *Campylobacter* on chicken carcasses during processing: A systemic review. *Poultry Science, 89*, 1070–1084.

Guldberg, P., Grønbæk, K., Aggerholm, A., Platz, A., thor Straten, P., Ahrenkiel, V., et al. (1998). Detection of mutations in GC-rich DNA by bisulphite denaturing gradient gel electrophoresis. *Nucleic Acids Research, 26*, 1548–1549.

Handley, J. A., Hanning, I., Ricke, S. C., Johnson, M. G., Jones, F. T., & Apple, R. O. (2010). Temperature and bacterial profile of post chill poultry carcasses stored in processing combo held at room temperature. *Journal of Food Science, 75*, M515–M520.

Handley, J. A., Park, S. H., Shi, Z., Dawoud, T. M., Kwon, Y. M., & Ricke, S. C. (2015). Chapter 6. *Salmonella* and the potential role for microbial process indicators on chicken carcasses. In S. C. Ricke, J. R. Donaldson, & C. A. Phillips (Eds.), *Food safety: Emerging issues, technologies and systems* (pp. 81–104). Oxford: Elsevier.

Handschur, M., Pinar, G., Gallist, B., Lubitz, W., & Haslberger, A. (2005). Culture free DGGE and cloning based monitoring of changes in bacterial communities of salad due to processing. *Food and Chemical Toxicology, 43*, 1595–1605.

Hanning, I. B., & Ricke, S. C. (2011). Chapter 11: Prescreening of microbial populations for the assessment of sequencing potential. In Y. M. Kwon & S. C. Ricke (Eds.), *High-throughput next generation sequencing* (pp. 159–170). New York: Springer.

Hayden, M. J., Nguyen, T. M., Waterman, A., & Chalmers, K. J. (2008). Multiplex-ready PCR: A new method for multiplexed SSR and SNP genotyping. *BMC Genomics, 9*, 80.

He, S., Zhou, Z., Liu, Y., Shi, P., Yao, B., Ringø, E., et al. (2009). Effects of dietary *Saccharomyces cerevisiae* fermentation product (DVAQUA®) on growth performance, intestinal autochthonous

bacterial community and non-specific immunity of hybrid tilapia (*Oreochromis niloticus*♀× O. *aureus*♂) cultured in cages. *Aquaculture, 294*, 99–107.

Heid, C. A., Stevens, J., Livak, K. J., & Williams, P. M. (1996). Real time quantitative PCR. *Genome Research, 6*, 986–994.

Hein, I., Flekna, G., Krassnig, M., & Wagner, M. (2006). Real-time PCR for the detection of *Salmonella* spp. in food: An alternative approach to a conventional PCR system suggested by the FOOD-PCR project. *Journal of Microbiological Methods, 66*, 538–547.

Hernández, M., Rodriguez-Lazaro, D., & Ferrando, A. (2005). Current methodology for detection, identification and quantification of genetically modified organisms. *Current Analytical Chemistry, 1*, 203–221.

Holland, P. M., Abramson, R. D., Watson, R., & Gelfand, D. H. (1991). Detection of specific polymerase chain reaction product by utilizing the 5′ → 3′ exonuclease activity of *Thermus aquaticus* DNA polymerase. *Proceedings of the National Academy of Sciences USA, 88*, 7276–7280.

Holst-Jensen, A., Rønning, S. B., Løvseth, A., & Berdal, K. G. (2003). PCR technology for screening and quantification of genetically modified organisms (GMOs). *Analytical and Bioanalytical Chemistry, 375*, 985–993.

Hoorfar, J., Ahrens, P., & Rådström, P. (2000). Automated 5′ nuclease PCR assay for identification of *Salmonella enterica*. *Journal of Clinical Microbiology, 38*, 3429–3435.

Horrocks, S. M., Anderson, R. C., Nisbet, D. J., & Ricke, S. C. (2009). Incidence and ecology of *Campylobacter* in animals. *Anaerobe, 15*, 18–25.

Houghton, S. G., & Cockerill, F. R. (2006). Real-time PCR: Overview and applications. *Surgery, 139*, 1–5.

Hume, M., Kubena, L., Edrington, T., Donskey, C., Moore, R., Ricke, S., et al. (2003). Poultry digestive microflora biodiversity as indicated by denaturing gradient gel electrophoresis. *Poultry Science, 82*, 1100–1107.

James, C., Vincent, C., de Andrade Lima, T. I., & James, S. J. (2005). The primary chilling of poultry carcasses—A review. *International Journal of Refrigeration, 29*, 847–862.

Jarquin, R., Hanning, I., Ahn, S., & Ricke, S. C. (2009). Development of rapid detection and genetic characterization of *Salmonella* in poultry breeder feeds. *Sensors, 9*, 5308–5323.

John, R., & Grody, W. W. (2008). Keeping up with the next generation: Massively parallel sequencing in clinical diagnostics. *The Journal of Molecular Diagnostics, 10*, 484–492.

Keener, K. M., Bashor, M. P., Curtis, P. A., Sheldon, B. W., & Kathariou, S. (2004). Comprehensive review of *Campylobacter* and poultry processing. *Comprehensive Reviews in Food Science and Food Safety, 3*, 105–116.

Keohavong, P., & Thilly, W. G. (1989). Fidelity of DNA polymerases in DNA amplification. *Proceedings of the National Academy of Sciences USA, 86*, 9253–9257.

Kim, J. S., Lee, G. G., Park, J. S., Jung, Y. H., Kwak, H. S., Kim, S. B., et al. (2007). A novel multiplex PCR assay for rapid and simultaneous detection of five pathogenic bacteria: *Escherichia coli* O157: H7, *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Vibrio parahaemolyticus*. *Journal of Food Protection, 70*, 1656–1662.

Kim, S. A., Park, S. H., Lee, S. I., & Ricke, S. C. (2017a). Development of a rapid method to quantify *Salmonella* Typhimurium using a combination of MPN with qPCR and a shortened time incubation. *Food Microbiology, 65*, 7–18.

Kim, S. A., Park, S. H., Lee, S. I., Owens, C. M., & Ricke, S. C. (2017b). Assessment of chicken carcass microbiome responses during processing in the presence of commercial antimicrobials using a next generation sequencing approach. *Scientific Reports, 7*, 43354. https://doi.org/10.1038/srep43354

Kong, R., Lee, S., Law, T., & Law, S. (2002). Rapid detection of six types of bacterial pathogens in marine waters by multiplex PCR. *Water Research, 36*, 2802–2812.

Koo, O., Mertz, A., Akins, E., Sirsat, S., Neal, J., Morawicki, R., et al. (2013). Analysis of microbial diversity on deli slicers using polymerase chain reaction and denaturing gradient gel electrophoresis technologies. *Letters in Applied Microbiology, 56*, 111–119.

Kowalchuk, G. A., Stephen, J. R., De Boer, W., Prosser, J. I., Embley, T. M., & Woldendorp, J. W. (1997). Analysis of ammonia-oxidizing bacteria of the beta subdivision of the class Proteobacteria in coastal sand dunes by denaturing gradient gel electrophoresis and sequencing of PCR-amplified 16S ribosomal DNA fragments. *Applied and Environmental Microbiology, 63*, 1489–1497.

Ku, C.-S., & Roukos, D. H. (2013). From next-generation sequencing to nanopore sequencing technology: Paving the way to personalized genomic medicine. *Expert Review of Medical Devices, 10*, 1–6.

Kuczynski, J., Lauber, C. L., Walters, W. A., Parfrey, L. W., Clemente, J. C., Gevers, D., et al. (2012). Experimental and analytical tools for studying the human microbiome. *Nature Reviews Genetics, 13*, 47–58.

Kwon, Y. M., & Ricke, S. C. (2011). *High-throughput next generation sequencing: Methods and applications*. New York: Humana Press.

Lai-Cheong, J. E., & McGrath, J. A. (2011). Next-generation diagnostics for inherited skin disorders. *The Journal of Investigative Dermatology, 131*, 1971–1973.

Lee, L. G., Connell, C. R., & Bloch, W. (1993). Allelic discrimination by nick-translation PCR with fluorogenic probes. *Nucleic Acids Research, 21*, 3761–3766.

Lee, Y., Moon, B., Park, J., Chang, H., & Kim, W. J. (2007). Expression of enterotoxin genes in *Staphylococcus aureus* isolates based on mRNA analysis. *Journal of Microbiology and Biotechnology, 17*, 461.

Lister, R., & Ecker, J. R. (2009). Finding the fifth base: Genome-wide sequencing of cytosine methylation. *Genome Research, 19*, 959–966.

Liu, D. P., Schmidt, C., Billings, T., & Davisson, M. T. (2003). Quantitative PCR genotyping assay for the Ts65Dn mouse model of down syndrome. *BioTechniques, 35*, 1170–1179.

Livak, K. J., Flood, S., Marmaro, J., Giusti, W., & Deetz, K. (1995a). Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization. *Genome Research, 4*, 357–362.

Livak, K. J., Marmaro, J., & Todd, J. A. (1995b). Towards fully automated genome–wide polymorphism screening. *Nature Genetics, 9*, 341–342.

Maciorowski, K. G., Herrera, P., Jones, F. T., Pillai, S. D., & Ricke, S. C. (2006). Cultural and immunological detection methods for *Salmonella* spp. in animal feeds—A review. *Veterinary Research Communications, 30*, 127–137.

Maciorowski, K. G., Pillai, S. D., Jones, F. T., & Ricke, S. (2005). Polymerase chain reaction detection of foodborne *Salmonella* spp. in animal feeds. *Critical Reviews in Microbiology, 31*, 45–53.

Mackay, I. M. (Ed.). (2007). *Real-time PCR in microbiology: From diagnosis to characterization*. Norfolk: Horizon Scientific Press.

Mafra, I., Ferreira, I. M., & Oliveira, M. B. P. (2008). Food authentication by PCR-based methods. *European Food Research and Technology, 227*, 649–665.

Mahon, J., Murphy, C., Jones, P., & Barrow, P. (1994). Comparison of multiplex PCR and standard bacteriological methods of detecting *Salmonella* on chicken skin. *Letters in Applied Microbiology, 19*, 169–172.

Mead, G. C. (2004). Current trends in the microbiological safety of poultry meat. *World's Poultry Science Journal, 60*, 112–118.

Meissner, A., Mikkelsen, T. S., Gu, H., Wernig, M., Hanna, J., Sivachenko, A., et al. (2008). Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature, 454*, 766–770.

Metzker, M. L. (2010). Sequencing technologies—the next generation. *Nature Reviews Genetics, 11*, 31–46.

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E. M., Kubal, M., et al. (2008). The metagenomics RAST server–a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics, 9*, 386.

Meyer, R. (1999). Development and application of DNA analytical methods for the detection of GMOs in food. *Food Control, 10*, 391–399.

Morin, R. D., Bainbridge, M., Fejes, A., Hirst, M., Krzywinski, M., Pugh, T. J., et al. (2008). Profiling the HeLa S3 transcriptome using randomly primed cDNA and massively parallel short-read sequencing. *BioTechniques, 45*, 81–94.

Morozova, O., & Marra, M. A. (2008). Applications of next-generation sequencing technologies in functional genomics. *Genomics, 92*, 255–264.

Moyer, C. L., Tiedje, J. M., Dobbs, F. C., & Karl, D. M. (1996). A computer-simulated restriction fragment length polymorphism analysis of bacterial small-subunit rRNA genes: Efficacy of selected tetrameric restriction enzymes for studies of microbial diversity in nature. *Applied and Environmental Microbiology, 62*, 2501–2507.

Muyzer, G., & de Waal, E. C. (1994). Determination of the genetic diversity of microbial communities using DGGE analysis of PCR-amplified 16S rDNA. In J. Lucas & P. Caumette (Eds.), *Microbial mats* (pp. 207–214). New York: Springer.

Muyzer, G., De Waal, E. C., & Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Applied and Environmental Microbiology, 59*, 695–700.

Muyzer, G., & Smalla, K. (1998). Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie Van Leeuwenhoek, 73*, 127–141.

Myers, R. M., Fischer, S. G., Lerman, L. S., & Maniatis, T. (1985). Nearly all single base substitutions in DNA fragments joined to a GC-clamp can be detected by denaturing gradient gel electrophoresis. *Nucleic Acids Research, 13*, 3131–3145.

Myers, R. M., Maniatis, T., & Lerman, L. S. (1987). Detection and localization of single base changes by denaturing gradient gel electrophoresis. *Methods in Enzymology, 155*, 501–527.

Nair, S., Lin, T. K., Pang, T., & Altwegg, M. (2002). Characterization of *Salmonella* serovars by PCR-single-strand conformation polymorphism analysis. *Journal of Clinical Microbiology, 40*, 2346–2351.

Neff, M. W., Broman, K. W., Mellersh, C. S., Ray, K., Acland, G. M., Aguirre, G. D., et al. (1999). A second-generation genetic linkage map of the domestic dog, *Canis familiaris*. *Genetics, 151*, 803–820.

Ng, S. B., Turner, E. H., Robertson, P. D., Flygare, S. D., Bigham, A. W., Lee, C., et al. (2009). Targeted capture and massively parallel sequencing of 12 human exomes. *Nature, 461*, 272–276.

Niesters, H. G. (2002). Clinical virology in real time. *Journal of Clinical Virology, 25*, 3–12.

Noverr, M. C., & Huffnagle, G. B. (2004). Does the microbiota regulate immune responses outside the gut. *Trends in Microbiology, 12*, 562–568.

Nübel, U., Engelen, B., Felske, A., Snaidr, J., Wieshuber, A., Amann, R. I., et al. (1996). Sequence heterogeneities of genes encoding 16S rRNAs in *Paenibacillus polymyxa* detected by temperature gradient gel electrophoresis. *Journal of Bacteriology, 178*, 5636–5643.

Oakley, B. B., Morales, C. A., Line, J. E., Seal, B. S., & Hiett, K. L. (2011). Application of high-throughput sequencing to measure the performance of commonly used selective cultivation methods for the foodborne pathogen *Campylobacter*. *FEMS Microbiology Ecology, 79*, 327–336.

Oakley, B. B., Morales, C. A., Line, J., Berrang, M. E., Meinersmann, R. J., Tillman, G. E., et al. (2013). The poultry-associated microbiome: Network analysis and farm-to-fork characterizations. *PLoS One, 8*(2), e57190. https://doi.org/10.1371/journal.pone.0057190

Oviedo-Rondón, E., Hume, M., Hernandez, C., & Clemente-Hernández, S. (2006). Intestinal microbial ecology of broilers vaccinated and challenged with mixed *Eimeria* species, and supplemented with essential oil blends. *Poultry Science, 85*, 854–860.

Oyarzabal, O. (2005). Reduction of *Campylobacter* spp. by commercial antimicrobials applied during the processing of broiler chickens: A review from the United States perspective. *Journal of Food Protection, 68*, 1752–1760.

Pacheco, L. G., Pena, R. R., Castro, T. L., Dorella, F. A., Bahia, R. C., Carminati, R., et al. (2007). Multiplex PCR assay for identification of *Corynebacterium pseudotuberculosis* from

pure cultures and for rapid detection of this pathogen in clinical samples. *Journal of Medical Microbiology, 56*, 480–486.

Panicker, G., Call, D. R., Krug, M. J., & Bej, A. K. (2004). Detection of pathogenic *Vibrio spp*. in shellfish by using multiplex PCR and DNA microarrays. *Applied and Environmental Microbiology, 70*, 7436–7444.

Park, S. H., Aydin, M., Khatiwara, A., Dolan, M. C., Gilmore, D. F., Bouldin, J. L., et al. (2014). Current and emerging technologies for rapid detection and characterization of *Salmonella* in poultry and poultry products. *Food Microbiology, 38*, 250–262.

Park, S. H., Hanning, I., Gilbert, W., Munro, M., Devareddy, L., & Ricke, S. C. (2013). Feeding mice aged and fresh blackberries powder supplements result in shifts in the gastrointestinal microflora. *Food Bioscience, 1*, 66–72.

Park, S. H., & Ricke, S. C. (2015). Development of multiplex and quantitative PCR assays for simultaneous detection of *Salmonella* genus, *Salmonella* subspecies I, *S*. Enteritidis, *S*. Heidelberg, and *S*. Typhimurium. *Journal of Applied Microbiology, 118*, 152–160.

Pedroso, A., Menten, J., Lambais, M., Racanicci, A., Longo, F., & Sorbara, J. (2006). Intestinal bacterial community and growth performance of chickens fed diets containing antibiotics. *Poultry Science, 85*, 747–752.

Plummer, E., Twin, J., Bulach, D., Garland, S., & Tabrizi, S. (2015). A comparison of three bioinformatics pipelines for the analysis of preterm gut microbiota using 16S rRNA gene sequencing data. *Journal of Proteomics and Bioinformatics, 8*, 283–291.

Polley, S. D., Boadi, S., Watson, J., Curry, A., & Chiodini, P. L. (2011). Detection and species identification of microsporidial infections using SYBR Green real-time PCR. *Journal of Medical Microbiology, 60*, 459–466.

Porcellato, D., Grønnevik, H., Rudi, K., Narvhus, J., & Skeie, S. (2012). Rapid lactic acid bacteria identification in dairy products by high-resolution melt analysis of DGGE bands. *Letters in Applied Microbiology, 54*, 344–351.

Postollec, F., Falentin, H., Pavan, S., Combrisson, J., & Sohier, D. (2011). Recent advances in quantitative PCR (qPCR) applications in food microbiology. *Food Microbiology, 28*, 848–861.

Qian, F., Chung, L., Zheng, W., Bruno, V., Alexander, R. P., Wang, Z., et al. (2013). Identification of genes critical for resistance to infection by West Nile virus using RNA-Seq analysis. *Viruses, 5*, 1664–1681.

Rajan, K., Shi, Z., & Ricke, S. C. (2017). Potential application of risk strategies for understanding *Salmonella* contamination in ground poultry products. *Critical Reviews Microbiology, 43*, 370–392.

Rehm, H. L. (2013). Disease-targeted sequencing: A cornerstone in the clinic. *Nature Reviews Genetics, 14*, 295–300.

Reis-Filho, J. S. (2009). Next-generation sequencing. *Breast Cancer Research, 11*, S12.

Repp, R., Rhiel, S., Heermann, K., Schaefer, S., Keller, C., Ndumbe, P., et al. (1993). Genotyping by multiplex polymerase chain reaction for detection of endemic hepatitis B virus transmission. *Journal of Clinical Microbiology, 31*, 1095–1102.

Ricke, S. C. (2003). Perspectives on the use of organic acids and short chain fatty acids as antimicrobials. *Poultry Science, 82*, 632–639.

Ricke, S. C., Dawoud, T. M., & Kwon, Y. M. (2015). Chapter 4. Application of molecular methods for traceability of foodborne pathogens in food safety systems. In S. C. Ricke, J. R. Donaldson, & C. A. Phillips (Eds.), *Food safety: Emerging issues, technologies and systems* (pp. 37–63). Oxford: Elsevier.

Ricke, S. C., Kundinger, M. M., Miller, D. R., & Keeton, J. T. (2005). Alternatives to antibiotics: Chemical and physical antimicrobial interventions and foodborne pathogen response. *Poultry Science, 84*, 667–675.

Rothrock, M. J., Locatelli, A., Glenn, T. C., Thomas, J. C., Caudill, A. C., Kiepper, B. H., et al. (2016). Assessing the microbiomes of scalder and chiller tank waters, throughout a typical commercial poultry processing day. *Poultry Science, 95*, 2372–2382.

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., et al. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science, 239*, 487–491.

Sails, A. D. (2013). Applications in clinical microbiology. In *Real-time PCR: Advanced technologies and applications* (pp. 123–158). Norfolk: Caister Academic Press.

Sanger, F., Donelson, J., Coulson, A., Kössel, H., & Fischer, D. (1973). Use of DNA polymerase I primed by a synthetic oligonucleotide to determine a nucleotide sequence in phage f1 DNA. *Proceedings of the National Academy of Sciences USA, 70*, 1209–1213.

Santegoeds, C. M., Nold, S. C., & Ward, D. M. (1996). Denaturing gradient gel electrophoresis used to monitor the enrichment culture of aerobic chemoorganotrophic bacteria from a hot spring cyanobacterial mat. *Applied and Environmental Microbiology, 62*, 3922–3928.

Saunders, C. J., Miller, N. A., Soden, S. E., Dinwiddie, D. L., Noll, A., Alnadi, N. A., et al. (2012). Rapid whole-genome sequencing for genetic disease diagnosis in neonatal intensive care units. *Science Translational Medicine, 4*, 154ra135.

Schadt, E. E., Turner, S., & Kasarskis, A. (2010). A window into third-generation sequencing. *Human Molecular Genetics, 19*, R227–R240.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., et al. (2009). Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology, 75*, 7537–7541.

Sevall, J. S. (1990). Detection of parvovirus B19 by dot-blot and polymerase chain reaction. *Molecular and Cellular Probes, 4*, 237–246.

Sheffield, V. C., Cox, D. R., Lerman, L. S., & Myers, R. M. (1989). Attachment of a 40-base-pair G+ C-rich sequence (GC-clamp) to genomic DNA fragments by the polymerase chain reaction results in improved detection of single-base changes. *Proceedings of the National Academy of Sciences USA, 86*, 232–236.

Smith, C. J., & Osborn, A. M. (2009). Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology. *FEMS Microbiology Ecology, 67*, 6–20.

Sofos, J. N., Flick, G., Nychas, G.-J., O'Bryan, C. A., Ricke, S. C., & Crandall, P. G. (2013). Chapter 6. Meat, poultry, and seafood. In M. P. Doyle & R. L. Buchanan (Eds.), *Food microbiology-fundamentals and frontiers* (4th ed., pp. 111–167). Washington: American Society for Microbiology.

Stratton, M. R., Campbell, P. J., & Futreal, P. A. (2009). The cancer genome. *Nature, 458*, 719–724.

Sunzeri, F., Lee, T.-H., Brownlee, R. G., & Busch, M. (1991). Rapid simultaneous detection of multiple retroviral DNA sequences using the polymerase chain reaction and capillary DNA chromatography. *Blood, 77*, 879–886.

Sutzko, M., & Widmann, S. (2017). The eight myths of pathogen testing in food and meat products. *International Food & Meat Topics, 28*, 25–26.

Teske, A., Sigalevich, P., Cohen, Y., & Muyzer, G. (1996). Molecular identification of bacteria from a coculture by denaturing gradient gel electrophoresis of 16S ribosomal DNA fragments as a tool for isolation in pure cultures. *Applied and Environmental Microbiology, 62*, 4210–4215.

Torpdahl, M., & Ahrens, P. (2004). Population structure of *Salmonella* investigated by amplified fragment length polymorphism. *Journal of Applied Microbiology, 97*, 566–573.

Towbin, J. A., Hejtmancik, J. F., Brink, P., Gelb, B., Zhu, X. M., Chamberlain, J. S., et al. (1993). X-linked dilated cardiomyopathy. Molecular genetic evidence of linkage to the Duchenne muscular dystrophy (dystrophin) gene at the Xp21 locus. *Circulation, 87*, 1854–1865.

Tucker, T., Marra, M., & Friedman, J. M. (2009). Massively parallel sequencing: The next big thing in genetic medicine. *The American Journal of Human Genetics, 85*, 142–154.

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature, 444*, 1027–1131.

Vallaeys, T., Topp, E., Muyzer, G., Macheret, V., Laguerre, G., Rigaud, A., et al. (1997). Evaluation of denaturing gradient gel electrophoresis in the detection of 16S rDNA sequence variation in rhizobia and methanotrophs. *FEMS Microbiology Ecology, 24*, 279–285.

Van den Bulcke, M., Lievens, A., Barbau-Piednoir, E., MbongoloMbella, G., Roosens, N., Sneyers, M., et al. (2010). A theoretical introduction to "Combinatory SYBR® Green qPCR Screening", a matrix-based approach for the detection of materials derived from genetically modified plants. *Analytical and Bioanalytical Chemistry, 396*, 2113–2123.

van der Luijt, R. B., Khan, P. M., Vasen, H. F., Tops, C. M., van Leeuwen-Cornelisse, I. S., Wijnen, J. T., et al. (1997). Molecular analysis of the APC gene in 105 Dutch kindreds with familial adenomatous polyposis: 67 germline mutations identified by DGGE, PTT, and southern analysis. *Human Mutation, 9*, 7.

Vandenvelde, C., Verstraete, M., & Van Beers, D. (1990). Fast multiplex polymerase chain reaction on boiled clinical samples for rapid viral diagnosis. *Journal of Virological Methods, 30*, 215–227.

VanGuilder, H. D., Vrana, K. E., & Freeman, W. M. (2008). Twenty-five years of quantitative PCR for gene expression analysis. *BioTechniques, 44*, 619.

Visel, A., Blow, M. J., Li, Z., Zhang, T., Akiyama, J. A., Holt, A., et al. (2009). ChIP-seq accurately predicts tissue-specific activity of enhancers. *Nature, 457*, 854–858.

Voelkerding, K. V., Dames, S. A., & Durtschi, J. D. (2009). Next-generation sequencing: From basic research to diagnostics. *Clinical Chemistry, 55*, 641–658.

Wang, L., Li, Y., & Mustapha, A. (2007). Rapid and simultaneous quantitation of *Escherichia coli* O157: H7, *Salmonella*, and *Shigella* in ground beef by multiplex real-time PCR and immunomagnetic separation. *Journal of Food Protection, 70*, 1366–1372.

Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: A revolutionary tool for transcriptomics. *Nature Reviews Genetics, 10*, 57–63.

Ward, D. M., Santegoeds, C. M., Nold, S. C., Ramsing, N. B., Ferris, M. J., & Bateson, M. M. (1997). Biodiversity within hot spring microbial mat communities: Molecular monitoring of enrichment cultures. *Antonie Van Leeuwenhoek, 71*, 143–150.

Wattel, E., Mariotti, M., Agis, F., Gordien, E., Prou, O., Courouce, A., et al. (1992). Human T lymphotropic virus (HTLV) type I and II DNA amplification in HTLV-I/II-seropositive blood donors of the French West Indies. *The Journal of Infectious Diseases, 165*, 369–372.

Wielen, P., Keuzenkamp, D., Lipman, L., Knapen, F., & Biesterveld, S. (2002). Spatial and temporal variation of the intestinal bacterial community in commercially raised broiler chickens during growth. *Microbial Ecology, 44*, 286–293.

Wilhelm, J., & Pingoud, A. (2003). Real-time polymerase chain reaction. *Chembiochem, 4*, 1120–1128.

Wirz, B., Tratschin, J.-D., Müller, H., & Mitchell, D. B. (1993). Detection of hog cholera virus and differentiation from other pestiviruses by polymerase chain reaction. *Journal of Clinical Microbiology, 31*, 1148–1154.

Wittwer, C. T., Herrmann, M. G., Moss, A. A., & Rasmussen, R. P. (1997). Continuous fluorescence monitoring of rapid cycle DNA amplification. *BioTechniques, 22*, 130–139.

Xuan, J., Yu, Y., Qing, T., Guo, L., & Shi, L. (2013). Next-generation sequencing in the clinic: Promises and challenges. *Cancer Letters, 340*, 284–295.

Ye, S., Dhillon, S., Ke, X., Collins, A. R., & Day, I. N. (2001). An efficient procedure for genotyping single nucleotide polymorphisms. *Nucleic Acids Research, 29*, e88–e88.

Ye, S., Humphries, S., & Green, F. (1992). Allele specific amplification by tetra-primer PCR. *Nucleic Acids Research, 20*, 1152–1152.

Yeh, S.-H., Tsai, C.-Y., Kao, J.-H., Liu, C.-J., Kuo, T.-J., Lin, M.-W., et al. (2004). Quantification and genotyping of hepatitis B virus in a single reaction by real-time PCR and melting curve analysis. *Journal of Hepatology, 41*, 659–666.

Yu, L., Zhang, W., Liu, L., & Yang, J. (2015). Determining microeukaryotic plankton community around Xiamen Island, southeast China, using Illumina MiSeq and PCR-DGGE techniques. *PLoS ONE, 10*, e0127721.

Chapter 12
Avian Pathogenic *Escherichia coli*: Link to Foodborne Urinary Tract Infections in Humans

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12.1 Extraintestinal Pathogenic *E. coli*: Major Human and Animal Pathogens

Escherichia coli is a highly versatile bacterial species, which exhibits substantial diversity in terms of physiology and metabolism (Kaper et al. 2004; Kohler and Dobrindt 2011; Leimbach et al. 2013). Most *E. coli* exist as a beneficial component of the commensal microbiota in the lower gastrointestinal tracts of humans and other vertebrates (Kaper et al. 2004; Leimbach et al. 2013; Croxen and Finlay 2010; Touchon et al. 2009). However, some *E. coli* strains have the potential to cause a spectrum of diseases in humans and animals (Russo and Johnson 2003; Kaper et al. 2004; Kohler and Dobrindt 2011; Leimbach et al. 2013). In the context of the host site of colonization, with potential progression to infection, two main categories of pathogenic *E. coli* have been recognized: diarrheagenic *E. coli* (DEC), which cause enteric infections, and extraintestinal pathogenic *E. coli* (ExPEC), which cause infections typically outside of the gastrointestinal tract (Russo and Johnson 2003; Kaper et al. 2004; Kohler and Dobrindt 2011). DEC are obligate pathogens and are usually introduced to the host via the oral-fecal route. They have been classified into at least six pathotypes on the basis of their virulence mechanisms: enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), and

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enteroinvasive *E. coli* (EIEC) (Kaper et al. 2004; Kohler and Dobrindt 2011; Leimbach et al. 2013; Russo and Johnson 2003; Croxen and Finlay 2010). Unlike DEC, ExPEC belong to the normal facultative intestinal flora of a subpopulation of healthy hosts (Kohler and Dobrindt 2011). ExPEC are subdivided into specific pathotypes based on the clinical syndromes they cause and their target host species. The most common ExPEC pathotypes include uropathogenic *E. coli* (UPEC), neonatal meningitis-causing *E. coli* (NMEC), septicemia-causing *E. coli* (SEPEC), and avian pathogenic *E. coli* (APEC) (Russo and Johnson 2003; Kaper et al. 2004; Kohler and Dobrindt 2011; Crossman et al. 2010; Croxen and Finlay 2010). Since ExPEC can cause infections in a variety of host species, ExPEC-induced infections result in a significant burden to society, public health, and animal agriculture (Foxman 2002, 2003).

ExPEC displays a high degree of genome heterogeneity and genetic diversity owing to the acquisition of unique virulence traits through horizontal gene transfer and other genetic modifications, such as gene loss and mutations (Leimbach et al. 2013; Touchon et al. 2009; Brzuszkiewicz et al. 2009). This genome plasticity bestows ExPEC with a unique ability to colonize and persist within a myriad of highly specialized ecological niches, such as the urinary tract, genital tract, respiratory tract, meninges, bloodstream, and mammary glands (Kaper et al. 2004; Pitout 2012; Smith et al. 2007; Dale and Woodford 2015) of humans and animals. In fact, these genetic modifications are also what make ExPEC distinct from DEC on the basis of genetic makeup and phylogeny (Leimbach et al. 2013). Although the immediate source of ExPEC is likely the host's colonic flora (Smith et al. 2007; Russo and Johnson 2003; Dale and Woodford 2015), the underlying mechanisms involved in ExPEC colonization, transmission dynamics, and clonal selection are largely unknown. However, ExPEC are the most abundant *E. coli* present in the intestinal flora of ~20% of healthy individuals (Johnson and Russo 2002), and in a study by Ejrnaes (2011), ≥10% of *E. coli* colonizing the intestinal tract was shown to possess ExPEC-associated virulence genes (Ejrnaes 2011). The emergence of hyper-virulent and multidrug-resistant strains of ExPEC constitutes an important public health issue (Pitout 2012; Petty et al. 2014; Russo and Johnson 2003; Dale and Woodford 2015; Riley 2014).

12.2 ExPEC Typing Techniques: The Key to Epidemiological Investigations

Rapid and accurate strain typing techniques are critical for effective surveillance, outbreak detection efforts, and understanding of the natural history of infection, pathogen transmission dynamics, virulence, lineages, and phylogeny of bacteria. In the post-genomic era, bacterial subtyping has largely shifted to genetic methods; however, ExPEC typing continues to utilize both phenotypic and genotypic techniques. Of these, the most widely used techniques include serotyping, plasmid profiling, virulence genotyping, antimicrobial resistance gene profiling, phylogenetic typing, multilocus sequence typing (MLST), CH typing, pulsed-field gel electrophoresis (PFGE), and whole genome sequencing (WGS).

Traditional serotyping of *E. coli* relies on the detection of the lipopolysaccharide (somatic or O) antigen, the capsular (K) antigen, and the flagellar (H) antigen in an agglutination assay (Kauffmann 1947; Orskov et al. 1977). Currently, *E. coli* O groups numbered O1–O188 have been defined, with 7 O groups (O31, O47, O67, O72, O93, O94, and O122) excluded and 4 O groups divided into subtypes (O18ab/ac, O28ab/ac, O112ab/ac, and O125ab/ac), resulting in a total of 186 O groups (Orskov et al. 1984; Scheutz et al. 2004). Despite the incredible diversity of ExPEC, a limited number of serogroups are known to be associated with specific clinical syndromes. For example, *E. coli* belonging to O1, O2, O4, O6, O14, O16, O22, O75, and O83 serogroups are responsible for more than 75% of UTIs (Johnson 1991; Stenutz et al. 2006), and *E. coli* belonging to O1, O2, O8, O35, and O78 serogroups are predominantly implicated in avian colibacillosis (Dho-Moulin and Fairbrother 1999; Ewers et al. 2007). However, serotyping alone is not adequate to differentiate ExPEC pathotypes or define the phylogenetic relatedness among strains (Ewers et al. 2007; Achtman and Pluschke 1986; Rodriguez-Siek et al. 2005a, b). While serogrouping provides epidemiologically important information, the cost, possible cross-reaction between different antigens, failure to designate an O type for some *E. coli* strains, and batch-to-batch variation of antisera, among other disadvantages, have limited the use of serotyping as the stand-alone typing technique for ExPEC. Recently, various molecular serotyping or genoserotyping schemes have been proposed in place of the traditional method (Fratamico et al. 2016; Ingle et al. 2016; Ballmer et al. 2007).

Typing based on plasmid profiling and plasmid replicon typing provides additional insight into the pathogenicity and antimicrobial resistance of ExPEC strains due to the fact that carriage of large plasmids harboring antimicrobial resistance genes and virulence genes is a characteristic trait of some ExPEC (Mellata et al. 2009, 2010; Tivendale et al. 2009a, b; Johnson et al. 2005c, 2006b, c, d, 2007b; Dobrindt 2005; Sorsa et al. 2003). However, plasmid typing also has some drawbacks. For example, plasmid profiles cannot distinguish between nonidentical plasmids of the same size. Plasmids can be lost from or acquired by the same strain over time or lost during bacterial storage and subculturing and some plasmids tend to delete or acquire DNA sequences resulting in erroneous profiles. Also, some plasmids, particularly, large- and low-copy plasmids, are difficult to isolate. Another limitation is that the ExPEC strain must contain at least one plasmid to be able to be typed by this method.

As mentioned above, ExPEC possess a diverse array of virulence genes, which form the basis for virulence genotyping. EXPEC virulence genes are most commonly present on plasmids or pathogenicity islands, which are large genetic regions acquired horizontally from other bacteria (Kaper et al. 2004; Leimbach et al. 2013; Dale and Woodford 2015). While considerable overlap exists among ExPEC pathotypes in terms of virulence gene profiles irrespective of the host origin, certain virulence genes are consistently more abundant in some of the pathotypes (Rodriguez-Siek et al. 2005a, b; Ewers et al. 2007). For example, *iss*, *tsh*, *sitD*, *hlyF*, and *iroN* predominantly occur in APEC; K1 capsular antigen gene, *ibeA*, and *gimB* island are common among NMEC; and group II capsular antigen gene is frequently present in both NMEC and UPEC (Rodriguez-Siek et al. 2005a, b; Johnson 1991; Smith et al. 2007; Belanger et al. 2011) (Table 12.1). Based on the commonality of certain genes

Table 12.1 Prevalence of common virulence-associated genes in ExPEC strains[a]

Gene or operon	Gene product or description	% Prevalence in ExPEC		
		APEC	UPEC	NMEC
Adhesins				
afa/draB	Afimbrial/Dr antigen-specific adhesin	1.3–8.2	6.1–12.6	3.8–27.1
fimH	Type 1 fimbrial adhesin	98.1	99.0	92.5–95.3
iha	Iron-regulated-gene-homologue adhesin	3.1–3.5	22.7–39.2	26.7–30.8
papA	Major subunit of pyelonephritis-associated pili or P-fimbriae	7.5	54.8	28.9–30.6
papC	Outer membrane usher protein of P-fimbriae	24.6–40.5	50.0–59.7	3.8–65.4
PapEF	Fimbrial tip components of P-fimbriae	39.2	55.4	32.2–32.9
papG allele I	Class I tip adhesin of P-fimbriae	1.5	0.6	6.7–56.5
papG allele II	Class II tip adhesin of P-fimbriae	40.7	42.9	22.2–31.4
papG allele III	Class III tip adhesin of P-fimbriae	0.7	20.2	4.4–21.6
sfa/foc	Common to S fimbriae and F1C fimbriae	4.4–8.8	26.4–50.0	26.9–55.3
sfaS	S fimbrial adhesin	4.0	14.1	46.7–49.4
tsh[b]	Temperature-sensitive hemagglutinin	52.7–54.9	2.6–4.5	11.5–32.9
Iron acquisition				
fyuA	Ferric *Yersinia* uptake (yersiniabactin receptor)	58.2–66.4	56.1–80.6	68.9–69.4
ireA	Iron-responsive element (putative catecholate siderophore receptor)	41.3–48.0	19.7–26.0	17.6–17.8
iroN[b]	Catecholate siderophore (salmochelin) receptor	83.7–87.4	34.8–72.7	63.3–69.2
irp2	Iron-repressible protein (yersiniabactin biosynthetic protein)	68.8	81.8	96.2
iutA[b]	Ferric aerobactin receptor	80.8	48.4	30.2–80.0
sitA[b]	Periplasmic iron-binding protein	89.6	83.4	92.5–96.5
sitD[c]	*Salmonella* iron transport system gene	31.6	56.1	69.2
sitD[b, d]	*Salmonella* iron transport system gene	73.2	21.2	42.3
Protectins/serum resistance				
cvaA[b]	Colicin V secretion protein	72.3–77.4	12.1–23.4	26.9–71.8
cvaC[b]	Colicin V synthesis protein	67.5	5.6	54.4–57.6
iss[b]	Increased serum survival	82.7–84.0	25.8–26.6	25.5–57.7
kpsMT I (K1)	Group I polysaccharide capsule synthesis	15.7	29.2	70.0–75.5
kpsMT II	Group II polysaccharide capsule synthesis	25.0	78.5	85.6–90.6
kpsMT III	Group III polysaccharide capsule synthesis	1.8	4.0	1.2–2.2
ompA	Outer membrane protein A	99.1	92.4	66.0–100.0
ompT[b, d]	Outer membrane protein T	81.6	5.6	64.4
ompT[c]	Outer membrane protein T	70.4	81.5	31.1
traT[b]	Transfer protein T	78.1–81.3	50.0–67.8	76.9–88.2

(continued)

Table 12.1 (continued)

Gene or operon	Gene product or description	% Prevalence in ExPEC		
		APEC	UPEC	NMEC
Toxins				
cnf1	Cytotoxic necrotizing factor 1	0.9–1.3	23.4–31.8	4.4–27.4
cdtB	Cytolethal distending toxin	1.1	8.7	35.6–37.7
hlyD	Transport protein for hemolysin A	0.9	34.1	3.3–35.9
hlyF[b]	Hemolysin F	75.4	5.6	30.2–62.4
sat	Secreted autotransporter toxin	0.4	21.2	34.6–49.0
vat	Vacuolating autotransporter toxin	33.4–39.8	54.5–62.3	50.0–77.6
Invasins				
gimB	Genetic island associated with neonatal meningitis	8.8–23.7	9.1–22.6	56.7–61.5
ibeA	Invasion of brain endothelium protein A	14.2–26.2	18.2–19.2	35.9–60.0
Miscellaneous				
etsA[b]	Putative ABC transport system	67.0	6.0	61.1–63.5
etsB[b]	Putative ABC transport system	66.8	6.0	58.9–61.2
malX (PAI)	Pathogenicity-associated island marker CFT073	11.2–15.0	33.3–68.2	7.7–57.6

[a]Virulence genes used and the gene prevalence rates observed are markedly different between studies. Results from studies performed evaluating relatively large isolate collections are presented (Johnson et al. 2008b; Logue et al. 2012; Wijetunge et al. 2015; Ewers et al. 2007)
[b]In APEC, the gene is often associated with large ColV plasmids
[c]Gene is located on the chromosome
[d]Gene is located on a plasmid

among all ExPEC, Johnson et al. (2005a, b, c) suggested a molecular definition for ExPEC isolates based on the presence of ≥2 genes out of 6 genes in a panel comprising *papA*, *papC*, *sfa/foc*, *afa/dra*, *kpsMII*, and *iutA* (Johnson et al. 2005b). However, an unambiguous set of virulence genes that can be utilized for reliable classification of all ExPEC into different pathotypes has yet to be identified (Ewers et al. 2007; Moulin-Schouleur et al. 2007; Dobrindt 2005; Kohler and Dobrindt 2011; Leimbach et al. 2013; Rodriguez-Siek et al. 2005a, b). Virulence genotyping techniques are usually rapid, easy, and high throughput, but different alleles or mutations in the virulence genes may hinder their detection.

In early studies, phylogenetic grouping of ExPEC used multilocus enzyme electrophoresis (MLEE) to classify *E. coli* into six groups, namely, A, B1, B2, C, D, and E, based on the electrophoretic polymorphism of esterases and some other enzymes (Ochman and Selander 1984a, b; Pupo et al. 1997; Selander et al. 1986; Goullet and Picard 1989). Later, MLEE was replaced by a rapid and inexpensive triplex polymerase chain reaction (PCR)-based phylogenetic typing scheme (Clermont et al. 2000; Gordon et al. 2008). This triplex PCR ordered *E. coli* into four phylogenetic groups (A, B1, B2, and D) based on the presence or absence of two genes (*chuA*, *yjaA*) and the DNA fragment, TSPE4.C2. According to this scheme, the significant human ExPEC isolates causing UTI, bacteremia, and meningitis were assigned mainly to the phylogenetic group B2 followed by group D, whereas a majority of

human commensal *E. coli* were catergorized into groups A and B1 (Smith et al. 2007; Rodriguez-Siek et al. 2005a; Johnson et al. 2001; Picard et al. 1999). Alternatively, most APEC belonged to groups A and D with only less than 20% of APEC were B2 *E. coli* (Rodriguez-Siek et al. 2005a; Barbieri et al. 2015; Ewers et al. 2009). On the contrary, Ewers et al. (2007) observed that most APEC belonged to groups A (46%) and B2 (35%) while only 17% were categorized into group D (Ewers et al. 2007). Despite the popularity of this method as a simple typing tool, some *E. coli* may not be assigned to one of the four groups, and common misassignments have been observed in the group A strains by the triplex PCR (Gordon et al. 2008). More recently, the historical phylogenetic typing was expanded to eight groups, A, B1, B2, C, D, E, F, and Clade 1, to include sensu stricto types and additional clades (Logue et al. 2017; Clermont et al. 2013). With the new scheme, a majority of human ExPEC were categorized into groups B2 and D, whereas a majority of APEC were reclassified under the phylogenetic group C, followed by F, B1, and B2 (Logue et al. 2017). Specifically, 53.8% of APEC were reclassified from A to C or D to E and F, while 9% of UPEC were recategorized to the newly introduced groups C, E, and F.

Multilocus sequencing typing (MLST) is another PCR-based assay commonly used for ExPEC typing. MLST assigns *E. coli* into sequence types (STs) and clonal complexes (CC) depending on the nucleotide sequence or allelic profiles of seven selected housekeeping genes (Maiden et al. 1998; Wirth et al. 2006). Of the different MLST schemes proposed for *E. coli* typing, the Achtman scheme is the most widely used method for ExPEC (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) (Achtman and Pluschke 1986; Clermont et al. 2015). The use of MLST has provided the opportunity to delineate clonal relationships among ExPEC. For example, based on MLST, numerous studies reported that certain STs, namely, ST38, ST131, ST405, and ST648, were successful ExPEC lineages and contributed to the global dissemination of antimicrobial resistance genes (Ewers et al. 2012; Pitout 2012; Hussain et al. 2012; Riley 2014). The use of MLST has repeatedly proved to be useful for studying epidemiological events that occur over a short period of time or geographical distance. However, the technique is relatively expensive and laborious due to PCR amplification and sequencing steps. Nevertheless, MLST neither differentiates between recombination events and point mutations nor detects the genetic changes occurring outside of seven selected housekeeping genes. It also lacks discriminatory power because of the slow accumulation of genetic variations across housekeeping genes and therefore is less suitable for routine typing of bacteria in outbreak investigations or local surveillance studies. In addition, *E. coli* strains categorized under the same ST may be dissimilar in terms of genetic properties, ecotype, and pathotype. The subsequently introduced CH typing or sequence analysis of *fumC* and *fimH* genes provides a better discriminatory power than MLST and delineates the clones within STs (Weissman et al. 2012). CH typing is remarkably less expensive and less laborious than MLST, but the use of only two genes for typing increases the likelihood of erroneous results due to recombination events.

Unlike the methods described above, PFGE scans the entire genome and is considered the "gold standard" typing method for many bacterial pathogens

(Ribot et al. 2006). Establishment of PulseNet, which is run by the Centers for Disease Control and Prevention (CDC), has provided an unprecedented opportunity for pathogen subtyping and outbreak investigation using PFGE (Ribot et al. 2006). Availability of standard operating procedures to generate accurate and reproducible results, acceptable discriminatory power, and utility for short-term epidemiological investigations has placed this method at the forefront of bacterial foodborne outbreak investigations. However, PFGE is a time-consuming and laborious technique, which requires skilled personnel to interpret the results. In addition, a single mutation can produce several differences in the banding pattern. PFGE is also unable to recognize genetic variations occurring outside of the corresponding restriction sites.

With the advent of next-generation sequencing technologies, WGS has become a powerful tool for ExPEC typing. The robustness of WGS rests with its ability to discern every aspect of the bacterial genome, including the serotype, virulence genes, plasmid profiles, antibiotic resistance genes, ST, and restriction enzyme maps. It also offers an opportunity to perform comparative genomics in order to identify single nucleotide polymorphisms (SNPs) as well as similarities/dissimilarities among ExPEC genomes (Price et al. 2013; Chen et al. 2013; Gomi et al. 2017; Ronco et al. 2017; Ingle et al. 2016). Although WGS provides high phylogenetic resolution, its utility has been limited at present due to the high cost and certain technical issues. WGS involves manipulation of metadata and computationally intense data analysis and interpretation, which require skilled personnel. However, increasing accessibility to bioinformatics tools via open-source servers and development of sophisticated algorithms and user-friendly analysis pipelines to augment assembly, annotation, and interpretation of data at the international level, as well as continued decreasing costs over time, will make WGS the preferred typing technique for ExPEC and other bacteria in the near future.

12.3 Urinary Tract Infections Due to UPEC: A Significant Healthcare Burden

Urinary tract infections (UTIs) are the most frequently diagnosed kidney and urologic diseases in humans, with an estimated 130–175 million cases occurring every year worldwide (Gupta et al. 2001; Flores-Mireles et al. 2015; Kucheria et al. 2005; Mehnert-Kay 2005). In developed countries, UTIs are one of the most common infectious syndromes, second only to respiratory tract infections in incidence (Hooton and Stamm 1997; Stamm and Hooton 1993). For example, in 2006, UTIs were responsible for over 11 million physician visits, 1.7 million emergency department visits, and 479,000 UTI-associated hospitalizations in the United States alone (DeFrances et al. 2008). Considering the fact that up to 50% patients with UTIs do not seek medical attention, these data are likely underestimates of the true medical burden of UTIs (Terlizzi et al. 2017; Foxman 2002). The incidence of UTIs is about four times higher in women than in men due to a number of factors, including the shorter length of the urethra and shorter distance between the anus and urethral

opening in women, as well as estrogen deficiency and vaginal atrophy in postmenopausal women (Foxman and Brown 2003). It is estimated that more than 50% of women will experience at least one episode of UTI in their lifetime and nearly one in three women will have had at least one episode of UTI requiring antimicrobial therapy by the age of 24 years. A significant proportion of women will also experience recurrent UTIs; sometimes, multiple episodes occur within 6 months of the initial infection (Ejrnaes 2011; Foxman 2010, 2014). Recurrent UTIs can be due to repeated introduction of the same strain or a new strain of UPEC into the bladder from the colon of the affected individual or reintroduction of the same strain already residing in the epithelium of the urinary bladder (Silverman et al. 2013; Ejrnaes 2011).

The majority of UTIs are due to *E. coli*, including up to 80–90% of community-acquired infections in ambulatory and hospitalized patients (Zhang and Foxman 2003; Warren 1996; Johnson and Stamm 1989; Ejrnaes 2011) and 25–35% of healthcare-associated infections (Donnenberg and Welch 1996; Foxman 2002; Bagshaw and Laupland 2006; Kucheria et al. 2005). These infections range from mild to life-threatening, manifesting as asymptomatic bacteriuria, urethritis, cystitis, renal abscesses, or pyelonephritis, with the possibility of scarring, acute kidney injury, and bacteremia with urosepsis (Johnson and Stamm 1989). As such, *E. coli* is also the most common cause of gram-negative bacteremia, with an estimated 50–70% of these infections originating from the urinary tract (Foxman, 2010, 2014; Litwin et al. 2005; Geerdes et al. 1992; Gransden et al. 1990). Clinically, UTIs can be classified as either uncomplicated or complicated based on the presence of structural or functional abnormalities in the urinary tract or the presence of certain comorbidities (e.g., diabetes mellitus, immunosuppression) (Foxman 2010). Uncomplicated UTIs occur in otherwise healthy individuals who are not pregnant and do not have structural or functional abnormalities or medical devices, such as catheters, in the urinary tract. It is estimated that 75% of uncomplicated UTIs and 65% of complicated UTIs are caused by UPEC strains (Foxman 2010).

Consequently, UPEC are associated with significant morbidity and mortality, as well as considerable healthcare costs worldwide, which are estimated in the billions of dollars annually. In 2000, UTIs led to more than $3.5 billion in evaluation and treatment-related costs (Litwin et al. 2005). If decreases in workforce productivity and associated morbidity of affected patients are taken into account, the true societal cost of UTIs is substantially higher than the estimated direct cost (Foxman 2010). Generally, UTIs are treated with a course of antibiotics; however, the increasing emergence of multidrug-resistant (MDR) strains of UPEC recently have led to limitations in effective antibiotic treatment options (Mellata 2013; Gupta et al. 2011; Pitout 2012; Riley 2014; Price et al. 2013). In addition, antibiotics can be associated with multiple adverse effects, including allergic reactions, gastrointestinal microbiome dysbiosis, and *Clostridium difficile* infection. Numerous studies have shown that UPEC are becoming increasingly resistant to fluoroquinolones, extended-spectrum beta-lactams, and trimethoprim/sulfamethoxazole, which are generally the most commonly used antibiotics for empiric treatment of UTIs (Cordoba et al. 2017; Allocati et al. 2013; Mellata 2013; Bonkat et al. 2013; Zhanel et al. 2005;

Schito et al. 2009; Hadifar et al. 2017; Woodford et al. 2007). Most importantly, a recent World Health Organization (WHO) report on antimicrobial resistance surveillance specified *E. coli* as one of the nine bacterial species of international concern among major species of bacteria causing community-acquired and healthcare-associated infections (WHO 2014).

Similar to other ExPEC, the immediate reservoir of UPEC is considered to be the colon of the affected individual (Gruneberg 1969; Johnson et al. 2003; Yamamoto et al. 1997; Spurbeck et al. 2012; Moreno et al. 2006; Foxman 2010). Although the original source of UPEC inhabiting the colon has yet to be determined, they either persist in the intestinal tract as commensals for a long period of time after entry or are introduced intermittently followed by transient colonization in the intestinal tract (Leimbach et al. 2013). Intriguingly, some lineages of UPEC referred to as "urovirulent clones" tend to be more common than other *E. coli* lineages owing to their enhanced fitness, host-to-host transmission, intestinal colonization, virulence, and antimicrobial resistance conferred by the genetic makeup (Achtman and Pluschke 1986; Bonacorsi et al. 2003; Banerjee and Johnson 2014; Moreno et al. 2006). A handful of studies have demonstrated that these urovirulent clones of *E. coli* are shared among family members, communities, or household pets, providing evidence for their transmission within a given household or community (Foxman et al. 1997, 2002; Johnson and Clabots 2006; Johnson et al. 2001; Murray et al. 2004). Although many studies have observed that the dominant *E. coli* population in the healthy intestines is represented by A and B1 phylogenetic groups (Smith et al. 2007; Barbieri et al. 2015; Ewers et al. 2009), some studies have reported conflicting results. For example, Zhang et al. (2002) found that the dominant *E. coli* in ~48% of healthy women, who had never experienced a UTI, belonged to the B2 group (Zhang et al. 2002). Similarly, Obata-Yasuoka et al. (2002) and, subsequently, Sannes et al. (2004) also reported B2 *E. coli* as the most common group of *E. coli* present in a majority of healthy adults (Sannes et al. 2004; Obata-Yasuoka et al. 2002). However, the group B2 *E. coli* recovered from UTIs and bacteremic cases contained a greater number of virulence genes than fecal B2 *E. coli* recovered from healthy adults (Sannes et al. 2004; Zhang et al. 2002).

UTIs have long been regarded as individual, isolated infections; however, some lineages of UPEC have been associated with multiple outbreaks in certain geographical regions indicating that at least some UPEC lineages were responsible for community-wide epidemics. For example, phylogenetic group B2 *E. coli* O25:H4-ST131 carrying the extended-spectrum beta-lactamase (ESBL) $bla_{\text{CTX-M-15}}$gene on an IncFII-type plasmid is globally disseminated (Clermont et al. 2008; Coque et al. 2008; Johnson et al. 2010, 2012a; Nicolas-Chanoine et al. 2008, 2014; Courpon-Claudinon et al. 2011; Karfunkel et al. 2013; Peirano et al. 2012; Manges et al. 2017; Colpan et al. 2013; Mathers et al. 2015) and was shown to be responsible for up to 60% of all *E. coli* infections, mainly UTI and urosepsis. Moreover, the same *E. coli* lineage was associated with 78% of infections caused by fluoroquinolone-resistant and/or ESBL-producing ExPEC (Johnson et al. 2010). Despite its global dissemination, *E. coli* ST131 appears to have emerged simultaneously and independently in various communities and unrelated areas of the globe with no discernable link among

patients (Mathers et al. 2015). The *fimH* gene analysis indicated that the most prominent lineage of *E. coli* ST131 was *H30*, which can be further divided into multiple sublineages with unique resistance profiles, namely, *H-30* (fluoroquinolone-susceptible and CTX-M negative), *H30-R* (fluoroquinolone-resistant and CTX-M negative), and *H30-Rx* (fluoroquinolone-resistant and CTX-M positive), based on WGS and phylogenetic SNP analysis (Banerjee et al. 2013; Johnson et al. 2015; Price et al. 2013). Alarmingly, Peirano et al. (2011) reported the isolation of carbapenem-resistant *E. coli* ST131 belonging to the B2 phylogenetic group from a patient with UTI who returned to the United States after being hospitalized in India (Peirano et al. 2011). In addition, a year-long community-acquired outbreak of cystitis, pyelonephritis, and septicemia due to MDR *E. coli* K52:H1, mostly serogroup O15, was reported from South London in the late 1980s (Phillips et al. 1988). Subsequently, *E. coli* O15:K52:H1 was linked to community-acquired UTIs in Denmark, Spain, and the United States (Dalmau et al. 1996; Prats et al. 2000; Manges et al. 2001; Johnson et al. 2002). Other clonal groups of *E. coli* implicated in potential community-wide outbreaks include O11/O77/O17/O73:K52:H18 in multiple states in the United States (Manges et al. 2001; Burman et al. 2003; Johnson et al. 2002), O78:H10 in Copenhagen, Denmark (Olesen et al. 1994), and CTX-M-14 ESBL-producing *E. coli* in Calgary, Alberta, Canada (Pitout et al. 2005).

Similar to other ExPEC, UPEC possess a number of virulence genes that encode for adhesins, toxins, iron acquisition systems, immune evasion mechanisms, and biofilm formation (Belanger et al. 2011; Ejrnaes 2011; Rodriguez-Siek et al. 2005a; Johnson 1991; Smith et al. 2007) (Table 12.1). Marrs et al. (2005) proposed that UPEC can be further divided into five subpathotypes based on the virulence gene repertoire (Marrs et al. 2005). Subsequently, Spurbeck et al. (2012) suggested that despite the UPEC virulence gene repertoire being highly diverse, a subset of *E. coli* harboring *yfcV*, *vat*, *fyuA*, and *chuA* possesses urovirulent properties, and, therefore, these four genes can be utilized to predict the uropathogenic potential of intestinal *E. coli* (Spurbeck et al. 2012).

12.4 APEC and Avian Colibacillosis: A Substantial Economic Burden to the Global Poultry Industry

APEC cause a variety of diseases in the avian host, including respiratory tract infection, septicemia, cellulitis, omphalitis, salpingitis, peritonitis, polyserositis, and septicemia, which are collectively termed as “avian colibacillosis” (Dho-Moulin and Fairbrother 1999; Nolan et al. 2003). Avian colibacillosis is responsible for significant financial losses for the poultry industry globally due to mortality, morbidity, reduced productivity, and carcass condemnation at slaughter (Nolan et al. 2003; Dho-Moulin and Fairbrother 1999; Dziva and Stevens 2008). Within the APEC pathotype, there are several subpathotypes in which each subpathotype is associated with a specific infectious syndrome (Olsen et al. 2012; Maturana et al. 2011). APEC can also be divided into two subgroups based on the presumed route of entry: the

respiratory tract infection followed by airsacculitis, perihepatitis, and pericariditis and the ascending infection, which results in salpingitis and/or peritonitis (Trampel et al. 2007). Infection by both routes has the potential to cause septicemia. Until recently, APEC were regarded as opportunistic pathogens residing in the intestinal tracts of birds, which are able to cause disease only when the host immunity is compromised due to stress, immunosuppression, coinfections, or poor management practices (Dho-Moulin and Fairbrother 1999; Nolan et al. 2003). However, recent genotyping studies indicated that some APEC carry a robust virulence factor armament and, hence, may possess the ability to behave as frank pathogens (Johnson et al. 2008a; Dziva and Stevens 2008; Rodriguez-Siek et al. 2005a, b). Conversely, APEC strains harboring fewer virulence traits may persist in the intestines of healthy birds as commensals and cause disease only when an opportunity arises (Rodriguez-Siek et al. 2005b; Dziva and Stevens 2008; Rodriguez-Siek et al. 2005a).

Because APEC infections are extraintestinal in nature, these *E. coli* are classified as ExPEC along with other *E. coli* that cause human extraintestinal infections, such as UTIs, neonatal meningitis, and septicemia (Kaper et al. 2004). The notable APEC belong to O1, O2, O18, and O78 serogroups, some of which are also the common serogroups implicated in human ExPEC infections, or are untypeable by traditional serotyping (Moulin-Schouleur et al. 2007; Johnson et al. 2008b). A recent Spanish study reported an increased prevalence of serogroup O111 among APEC isolated between 2004 and 2011 compared to APEC isolated during the period from 1991 to 2000, indicating the possibility of detecting previously unidentified APEC serogroups (Mora et al. 2012). They also observed an increasing prevalence of fluoroquinolone-resistant strains of APEC belonging to two related highly virulent clonal groups, O111:H4:D-ST2085 and O111:H4-D-ST117 (Mora et al. 2012). Interestingly, ST117 included *E. coli* belonging to many serotypes and has been isolated from human UTI cases, chickens with colibacillosis, and retail poultry (Vincent et al. 2010; Ozawa et al. 2010). For example, ST117 UPEC *E. coli* isolated from Canada belonged to two different serotypes (O114:H4 and O24:NM), whereas ST117 retail poultry isolates belonged to seven serotypes (O24:H4, O45:H4, O53:H4, O114:H4, O143:H4, O160:H4, and ONT:HNM) (Vincent et al. 2010). A Japanese study recovered ST117 APEC belonging to O78 serogroup from broiler and layer chickens diagnosed with colibacillosis (Ozawa et al. 2010).

Although the APEC pathotype has yet to be fully defined, many traits, such as adhesins, serum resistance factors, iron acquisition factors, and toxins, have been implicated in APEC virulence (Rodriguez-Siek et al. 2005b) (Table 12.1). Some of the virulence genes, along with antimicrobial resistance genes, tend to occur on large conjugative ColV plasmids (Johnson et al. 2004, 2005c, 2006b, c, d; Rodriguez-Siek et al. 2005b; Tivendale et al. 2009a; Mellata et al. 2009), and the possession of these plasmids is strongly linked to APEC virulence (Johnson et al. 2008b; Mellata et al. 2010, 2012; Tivendale et al. 2009b; Skyberg et al. 2006, 2008). Johnson et al. (2008a) reported that five ColV plasmid genes, namely, *iroN*, *OmpT*, *hlyF*, *iss*, and *iutA*, were more prevalent in APEC than in *E. coli* isolated from feces of apparently healthy birds (avian fecal *E. coli* or AFEC) and indicated that this gene panel can be used to predict the APEC pathotype (Johnson et al. 2008a). Nevertheless, some

large non-ColV-type plasmids which were not directly involved in APEC virulence played a critical role in APEC persistence and survival in various hostile niches (Mellata et al. 2012). Although ColV gene-based pentaplex PCR could differentiate a significant proportion of APEC strains from AFEC, most APEC clustered together with human ExPEC based upon virulence gene content implying that APEC may possess zoonotic potential (Johnson et al. 2008b, 2017). Because the studies on APEC virulence genes have largely relied on the genes that are known to be associated with the virulence of human ExPEC, it is conceivable that the discovery of hitherto unknown virulence genes may pave the way toward distinctly defining the APEC pathotype in the future. Some APEC strains also carry large transmissible multidrug resistance plasmids which confer resistance to various antimicrobial agents, including antibiotics and disinfectant heavy metals (Johnson et al. 2006d; Fernandez-Alarcon et al. 2011) making the treatment and control of these infections extremely difficult. Johnson et al. (2012b), by examining a large collection of APEC, AFEC, UPEC, NMEC, human fecal *E. coli*, and human vaginal *E. coli*, observed that multidrug resistance and the plasmids and mobile elements encoding for multidrug resistance were more prevalent in avian *E. coli* than their corresponding human counterparts (Johnson et al. 2012b). These researchers also noticed that multidrug resistance was more widespread in APEC than in AFEC and that multidrug resistance was mostly correlated with the possession of IncA/C, IncP1-α, IncF, and IncI1 plasmid types (Johnson et al. 2012b).

12.5 Retail Poultry *E. coli* (RPEC): An Understudied Foodborne Pathogen?

Poultry meat constitutes a great source of human proteins, but it also serves as an important vehicle for the transfer of pathogenic bacteria, antibiotic-resistant bacteria, and antibiotic resistance genes to consumers through the food chain. Studies from many countries have reported a high prevalence of *E. coli,* including ExPEC, in poultry meat (Jakobsen et al. 2010a, b, c; Johnson et al. 2005a, b; Bonnet et al. 2009; Koga et al. 2015; Lyhs et al. 2012; Mitchell et al. 2015; Lima-Filho et al. 2013; Wu et al. 2014) highlighting possible foodborne transmission of ExPEC to humans. In general, most retail poultry meat *E. coli* and *E. coli* isolated from chicken carcasses belong to phylogenetic groups A or D. However, a substantial proportion (12–21%) of RPEC has been grouped under B2, which is known to contain potent human and animal ExPEC (Johnson et al. 2009; Kobayashi et al. 2011). Interestingly, a study carried out in Spain found that 60% of RPEC from retail chicken and turkey meat were group A/ST131 or B1 while no B2 *E. coli* were detected (Egea et al. 2012). A recent study by Johnson et al. (2017) which sampled raw chicken breasts from retail stores in 26 states in the United States reported that MDR *E. coli* possessing ExPEC characteristics, including B2/ST131 clonal type, were present in both regular meat and products labeled "organic" (Johnson et al. 2017). Although virulent properties were equally present in *E. coli* recovered from both organic and

nonorganic chicken breast types, *E. coli* from products labeled "organic" were less extensively antibiotic resistant than *E. coli* isolated from regular products.

Many studies have shown that multidrug-resistant isolates of *E. coli*, ESBL-producing *E. coli*, and AmpC beta-lactamase-producing *E. coli* are widespread in poultry meat around the globe (Egea et al. 2012; Aliyu et al. 2016; EUCAST 2011; Ghodousi et al. 2015; Lim et al. 2016; Pehlivanlar Onen et al. 2015; Le et al. 2015; Sheikh et al. 2012; Cortes et al. 2010; Adeyanju and Ishola 2014). A Canadian study, examining retail meat samples purchased from 2007 to 2008 in Alberta, Canada, found that $bla_{\text{CMY-2}}$ was more prevalent in retail chicken than in other meat types (Sheikh et al. 2012). In Spain, the prevalence of retail meat colonized by ESBL-producing *E. coli* increased from 62.5% in 2007 to 93.3% in 2010 (Egea et al. 2012). Likewise, a Dutch study indicated that 12% of retail chicken samples were contaminated with AmpC beta-lactamase-producing *E. coli* and that $bla_{\text{CMY-2}}$, which confers this resistance, was located on IncK- (91% isolates) or IncI1-type (9% isolates) plasmids (Voets et al. 2013). The IncK plasmids were also widespread among AmpC beta-lactamase-producing *E. coli* from Danish and imported broiler meat in Denmark (Hansen et al. 2016). The $bla_{\text{CMY-2}}$-positive IncK- and IncI1-type *E. coli* plasmids from humans, poultry meat, poultry, and dogs in Denmark were not associated with a specific *E. coli* genetic background indicating that plasmid horizontal transfer is more important than clonal expansion for the transmission of $bla_{\text{CMY-2}}$-mediated cephalosporin resistance from animals to humans and vice versa (Hansen et al. 2016). Intriguingly, despite extremely low antibiotic usage by the Norwegian broiler industry (NORM/NORM-VET 2011, 2012, 2014), about one-third of *E. coli* recovered from broilers and retail chicken fillets produced in Norway during the period from 2011 to 2014 were cephalosporin-resistant (NORM/NORM-VET 2011, 2012, 2014). Subsequent studies noted that retail chicken cephalosporin-resistant *E. coli* had remarkably low genetic diversity, mainly limited to two STs and phylogenetic group D, and typically carried the $bla_{\text{CMY-2}}$ gene on an IncK-type plasmid (Mo et al. 2016). Unlike IncK plasmids mentioned in the Danish study (Hansen et al. 2016), both clonal dissemination and horizontal transfer were shown to be important for the dissemination of Norwegian $bla_{\text{CMY-2}}$ IncK plasmid-mediated cephalosporin resistance (Mo et al. 2016).

Johnson et al. (2005a) reported that *E. coli* recovered from poultry meat differed from produce-source *E. coli* with respect to phylogenetic background (Johnson et al. 2005a), suggesting that a specific subset of *E. coli* capable of withstanding decontamination steps during meat processing may continue to remain on poultry meat as RPEC. A subsequent study by Johnson et al. (2009) compared a collection of APEC with AFEC, *E. coli* isolated from crop and gizzard (CGEC), and RPEC for their serogroups, virulence genes, and phylogenetic groups (Johnson et al. 2009). Most interestingly, they observed that RPEC were more similar to APEC and CGEC than they were to AFEC, indicating that fecal contamination is not the only route of *E. coli* contamination of poultry meat. Perhaps, certain virulence, fitness, and/or resistance traits of CGEC necessary for *E. coli* survival in the crop/gizzard are also required for *E. coli* survival during poultry processing and their survival/persistence on poultry meat.

12.6 APEC as a Foodborne Source of Human UTIs: Making the Connection

It was originally thought that APEC and UPEC were two distinct subtypes of ExPEC, with APEC causing disease only in the avian host and UPEC causing UTIs in the mammalian host. However, recent studies have provided substantial evidence for an association between APEC and human UTI. If such a connection exists, the most compelling hypothesis is that poultry meat serves as a food reservoir of UPEC which leads to subsequent colonization in the lower intestinal tract of humans, a source of APEC genes that makes intestinal *E. coli* urovirulent and antibiotic resistant, and/or a vehicle for foodborne transmission of *E. coli* causing UTIs. This association has been prepositioned as a result of experimental and epidemiological observations, which have revealed the following: (1) the ExPEC genome, like any other *E. coli* genome, is highly plastic in that DNA can be acquired or lost, leading to modification of the genome (Leimbach et al. 2013; Touchon et al. 2009; Brzuszkiewicz et al. 2009); (2) a close relationship among human UPEC, APEC, and RPEC with respect to serogroups, phylogenetic groups, virulence genotypes, antibiotic resistance profiles, virulence gene transcriptional profiles, whole genome comparisons, and/or in vitro and in vivo pathogenicity evaluation studies (Mellata et al. 2009, 2018; Johnson et al. 2005b, 2017; Moulin-Schouleur et al. 2007; Lima-Filho et al. 2013; Giufre et al. 2012; Jakobsen et al. 2010d, 2012; Kluytmans et al. 2013; Toth et al. 2012; Literak et al. 2013); (3) APEC can be transmitted to the human host (Jakobsen et al. 2010a, b), and APEC plasmids and genes can be acquired by commensal *E. coli* or UPEC (Skyberg et al. 2006; Levy et al. 1976; Nolan et al. 2003); (4) epidemic strains of UPEC have been implicated in a number of community-based outbreaks of uncomplicated UTIs in the absence of a known common source (Riley 2014; Price et al. 2013; Clermont et al. 2008; Coque et al. 2008; Johnson et al. 2010, 2012a; Nicolas-Chanoine et al. 2008, 2014; Courpon-Claudinon et al. 2011; Karfunkel et al. 2013; Peirano et al. 2012; Manges et al. 2017; Colpan et al. 2013; Mathers et al. 2015); (5) a high resemblance of some epidemic strains of UPEC to RPEC (Platell et al. 2011; Vincent et al. 2010; Ghodousi et al. 2016); and (6) a linkage between retail meat consumption and intestinal colonization by antimicrobial-resistant UPEC (Manges et al. 2007).

Given that the immediate source of UPEC for UTIs is the affected individual's own colonic flora, the most plausible route of UPEC introduction to the human colon is via the oral route. In that process, food is a realistic vehicle for *E. coli* transmission to the human intestinal tract. Upon colonization, APEC may persist in the intestinal tract as urovirulent *E. coli* or act as a source of virulence genes and/or antibiotic resistance genes for otherwise harmless intestinal *E. coli*, subsequently conferring them the ability to cause UTIs. Along these lines, previous studies have shown that human volunteers who consumed a sterile diet for several weeks had only a restricted number of *E. coli* serotypes (Bettelheim et al. 1977) and antimicrobial-resistant *E. coli* (Corpet 1988) in the intestinal microbiota suggesting that food may serve as a source of microbes and microbial genes thereby increasing

the diversity of the intestinal microbial population. As extraintestinal pathogens, both UPEC and APEC encounter similar challenges during the infection process and, therefore, are likely to carry similar genetic architectures. In fact, studies conducted in the United States and other geographic regions have reported close relatedness between UPEC and APEC with respect to serogroups, virulence gene profiles, and phylogenetic groups (Ewers et al. 2007; Rodriguez-Siek et al. 2005a; Zhao et al. 2009; Maluta et al. 2014; Nandanwar et al. 2014). Additionally, WGS and comparative genomics approaches have repeatedly shown a high degree of genetic similarity between APEC and UPEC, suggesting that at least some of these strains possess the genetic sustenance required to cross the host-species barrier (Bauchart et al. 2010; Kariyawasam et al. 2007). A study by Vincent and colleagues (Vincent et al. 2010) demonstrated that O25:H4-ST131 and O114:H4-ST117 RPEC were indistinguishable or closely related to *E. coli* isolated from women with UTIs during the same sampling period and from the same geographic region, on the basis of phenotypic and genotypic properties (Vincent et al. 2010). In a similar investigation, Ghodousi et al. (2016) detected *E. coli* ST131 *H30-R* and ST131 *H30-Rx* subclones in retail chicken meat in Italy by examining a collection of fluoroquinolone-resistant and AmpC/ESBL-producing *E. coli* isolated from processed retail meat from 2013 to 2015 (Ghodousi et al. 2016). However, antibiotic-resistant B2/ST131 ExPEC-like *E. coli* recovered from retail chicken breasts in the United States in 2013 were shown to belong to the ST131 *H22* clonal subset but not to the ST131 *H30* pandemic lineage (Johnson et al. 2017). In addition to previously discussed resistance traits, plasmid-mediated *mcr-1*, which confers resistance to colistin, was also detected in ESBL-producing *E. coli* recovered from retail chicken meat in the Netherlands (Kluytmans-van den Bergh et al. 2016), China (Liu et al. 2016), and Denmark (Hasman et al. 2015).

Besides the genetic evidence, in vitro and in vivo experimental infection models also provide substantial evidence for a possible link between avian *E. coli* and human UTI. For example, acquisition of a large conjugative ColV plasmid of APEC by a commensal *E. coli* isolate enhances its abilities to kill 12-day-old chicken embryos, grow in human urine, and colonize the murine kidney in a model of ascending UTI, indicating that the plasmid is not only involved in urovirulence but also transmissible from APEC to other *E. coli*, rendering them uropathogenic (Skyberg et al. 2006, 2008). In another study, human ExPEC and APEC belonging to ST95, which is also one of the most dominant lineages of human ExPEC and APEC, were equally competent in adhering to and invading two mammalian kidney cell lines, forming strong biofilms in M63 medium, and resisting bactericidal effects of human and avian serum (Nandanwar et al. 2014). Moreover, in a chicken challenge model, in which 1-day-old chicks were challenged by the air sac route with either UPEC strain U17 or APEC strain E058, possessing similar virulence gene profiles, both strains had similar LD_{50} demonstrating that UPEC has the ability to cross the host-species barrier and cause disease in chickens (Zhao et al. 2009). When the same challenge models (chicken air sac or murine UTI models) were used to study transcriptional profiles of APEC E058 and UPEC U17 on the basis of 152 genes common to both, the gene expression profiles of UPEC and APEC demonstrated a similar pattern (Zhao et al. 2009).

Strikingly, there was no difference in the transcriptional profiles of human ExPEC strain IHE3034 and APEC strain BEN374 at both 37 °C (human body temperature) and 42 °C (avian body temperature), revealing that a common set of genes are important in causing disease in both human and avian hosts (Bauchart et al. 2010). Apart from APEC isolated from birds with colibacillosis, Stromberg et al. (2017) recently reported that some AFEC also possessed phenotypic and genotypic characteristics pertaining to UPEC or APEC and were able to cause UTI and sepsis in mice and colibacillosis in chickens, therefore demonstrating that healthy chickens can serve as a reservoir for human ExPEC (Stromberg et al. 2017). This study reiterated the zoonotic risk posed by healthy chickens since fecal *E. coli* will not only contaminate poultry meat but will also disseminate over a broader area and contaminate the environment, including soil, water, and produce (Fig. 12.1).

Regardless of the aforementioned resemblance, a majority of APEC and human ExPEC, including UPEC, possess subtype-specific profiles and segregate into discrete groups according to virulence gene profile-based cluster analysis, indicating that not all APEC are capable of causing UTI and vice versa (Maluta et al. 2014; Johnson et al. 2008b). Nevertheless, a small subset of human ExPEC and APEC clustered together, suggesting that ExPEC in this mixed cluster may have the propensity to cause disease in both hosts and, therefore, may pose a zoonotic risk (Moulin-Schouleur et al. 2007; Johnson et al. 2008b; Bidet et al. 2007). For example, Moulin-Schouleur et al. (2007) found that this mixed subcluster was represented by UPEC, NMEC, and APEC belonging to the serogroups O1, O2, or O18 and phylogenetic group B2 (subcluster B2-1). Irrespective of the host origin, all of these *E. coli* were highly virulent in chickens and possessed $fimA_{MT78}$, *neuC*, *iutA*, *ibeA*, *tsh, cdt*,

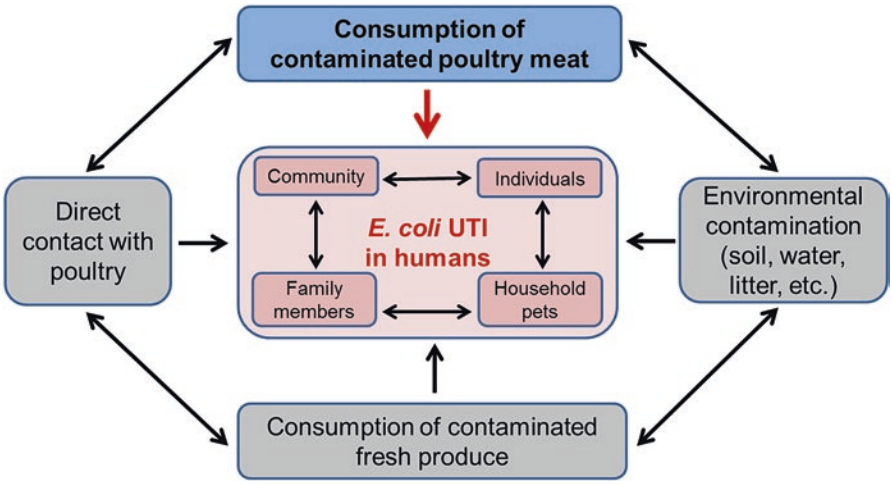

Fig. 12.1 Hypothetical modes of transmission of APEC bacteria and APEC plasmids and genes to humans in community-acquired UTIs. While there are numerous pathways, transmission of APEC via consumption of poultry meat as a potential source of UTIs in the community is one of the greatest concerns, as discussed in this chapter

and *hlyF*. A subsequent study reported that human and avian ExPEC in a mixed cluster consisted of phylogenetic group B2/ribotype $B2_1$/ST29 *E. coli* belonging to O1:K1, O2:K1, O18:K1, and O45:K1 serotypes (Bidet et al. 2007). All these *E. coli* appeared to carry the specific virulent subgroup (*svg*) locus (Bidet et al. 2007). These observations were further supported by a study by Johnson et al. (2008b), which examined a large collection of UPEC, NMEC, and APEC to report that the majority of isolates within the mixed cluster belonged to O1, O2, or O18 serogroups, the ST95 clonal group, and the B2 phylogenetic group (Johnson et al. 2008b). Nearly all of these *E. coli* contained ColV PAI-associated genes, and ~58% harbored *svg*. Interestingly, *svg* + B2 *E. coli* that represented the mixed cluster were recovered from retail poultry meat products in Finland (Lyhs et al. 2012; Johnson et al. 2007a). Later, Danzeisen et al. (2013) further characterized 88 isolates selected from a large collection representing human (UPEC, NMEC, HFEC) and avian-source (APEC, AFEC, and CGEC) *E. coli* and showed that clonal complexes with host source overlap included ST95, ST23, and some novel clonal groups (Danzeisen et al. 2013).

Johnson et al. (2006a, 2007a) found human fecal *E. coli*, and RPEC that were resistant to trimethoprim-sulfamethoxazole, fluoroquinolones, and extended-spectrum cephalosporins were highly similar to each other in terms of phylogenetic groups and virulence markers, while antibiotic-susceptible human fecal *E. coli* and APEC differed significantly (Johnson et al. 2007a; Johnson et al. 2006a). Further, there was no difference in the genetic backbones among ESBL-producing *E. coli* derived from retail chicken meat, feces of clinically healthy humans, or humans with septicemia as determined by MLST, restriction fragment length polymorphism (RFLP), and PFGE, suggesting an association between ESBL-producing RPEC and human fecal *E. coli* (Kluytmans et al. 2013). Similarly, a Norwegian study found that extended-spectrum cephalosporin-resistant ST38 RPEC and UPEC were remarkably similar to each other, with fewer than 15 SNP differences based on WGS and SNP analysis (Berg et al. 2017). In addition to overall strain resemblances, the IncK/bla_{CMY-2} plasmid variants of UPEC and avian isolates also demonstrated a high degree of similarity, indicating a possible clonal transfer of cephalosporin-resistant *E. coli* from chicken meat to humans and subsequent antibiotic-resistant *E. coli* UTIs. It is likely that poultry meat may serve as a conduit for transfer of AmpC-resistance plasmids from avian *E. coli* to human intestinal microbiota (Berg et al. 2017).

In an attempt to provide direct evidence for meat and production animals as a source of *E. coli* B2 strains in the intestines of UTI patients and community-dwelling humans, Jakobsen et al. (2010d, 2011) examined a large collection of geographically and temporally matched *E. coli* isolates from UTI patients, community-dwelling humans, production animals, and fresh meat in Denmark using a microarray approach (Jakobsen et al. 2010d, 2011). By profiling 315 virulence genes and 82 antimicrobial resistance genes, they observed that UTI and community-dwelling human isolates frequently clustered together with meat-source and animal-source *E. coli* and, most strikingly, some B2 *E. coli* strains from UTI and meat had identical gene profiles. Subsequent studies conducted by the same investigators reported that the B2 *E. coli* recovered from meat and healthy animals were able to cause UTI in a mouse model of ascending UTI, providing strong evidence for the zoonotic potential

of RPEC (Jakobsen et al. 2010a, b, 2012). However, when the ExPEC-specific PCR panel (*papA*, *papC*, *sfa/foc*, *afa/dra*, *kpsMII*, and *iutA*) was applied, *afa* was not detected in meat and animal isolates, suggesting the presence of an additional external source of virulence genes in community-dwelling humans and UTI patients (Jakobsen et al. 2010d). Interestingly, in a case-control study by Manges et al. (2007), women with multidrug-resistant *E. coli* UTIs and ampicillin- or cephalosporin-resistant *E. coli* UTIs reported more frequent consumption of chicken and pork, respectively, than women with UTIs caused by fully susceptible *E. coli* (Manges et al. 2007), providing epidemiologic evidence for foodborne transmission of antimicrobial-resistant UPEC to humans. Most recently, Mellata et al. (2018) demonstrated that chicken meat and egg shell *E. coli* strains containing dissimilar phenotypic traits were capable of causing UTI, sepsis, and meningitis in murine models of infection, suggesting that avian-source *E. coli* with various genetic and phenotypic backgrounds possess zoonotic potential (Mellata et al. 2018). They also noted that the strain's ability to swim on soft agar plates and form biofilms in human urine was correlated with its ability to cause UTI and sepsis, respectively (Mellata et al. 2018).

12.7 Concluding Remarks

Given the continuing global increase in community-acquired UTIs due to MDR strains of UPEC, there is an urgent need to identify possible sources of urovirulent *E. coli* involved in such infections. A growing body of literature suggests a foodborne link between APEC and UTIs caused by *E. coli* (Manges 2016; Smith et al. 2007; Mellata 2013; Markland et al. 2015; Belanger et al. 2011). Despite the lack of direct evidence, experimental and epidemiological studies have provided substantial corroboration to support such an association. According to the prevailing foodborne hypothesis, *E. coli* enter the human host via consumption of contaminated poultry meat. Those that are successful colonizers with the correct virulence armament will then persist in the human intestines as "urovirulent clones" causing single or multiple episodes of UTI. These exogenously acquired *E. coli* may also transfer their antibiotic resistance genes, virulence genes, and resistance/virulence plasmids to endogenous intestinal microflora, contributing to the emergence of antibiotic-resistant pathogenic bacteria. Unlike UTIs caused by *E. coli* present in the intrinsic flora, urovirulent *E. coli* transmitted to humans from food sources has the propensity to cause widespread epidemiologic disease, leading to a significant healthcare and societal burden of UTIs, in particular, due to global trade practices. The current scientific literature suggests that not all APEC has the ability to cause UTI but, rather, that a specific subset belonging to the B2 phylogenetic group exhibits urovirulent properties. It is likely that *E. coli* in this subset harbor not only the virulence and fitness genes required to cause infection but also a gene repertoire needed to overcome various barriers that they may encounter during poultry slaughtering and meat processing and transport. Although this chapter focuses on poultry meat as a

foodborne source of APEC bacteria for human UTI and APEC genes to humans, these *E. coli* are typically present in the intestines of poultry and are passed through feces, resulting in a broader dissemination via contamination of the environment, including water and poultry litter. Therefore, fresh produce fertilized with poultry litter may be another conduit for foodborne transmission of APEC and their genes to humans.

Discerning a direct relationship between foodborne transmission of urovirulent *E. coli* and development of UTIs in humans has been hindered, at least in part, due to an unclear duration between *E. coli* acquisition in the human intestine and onset of infection. To this end, mathematical models and epidemiological simulations may provide a useful framework in which to understand the nuances of RPEC transmission dynamics causing UTI. Although additional research is necessary to confirm a link between APEC and human UTIs, a potential poultry reservoir of UPEC reinforces the need for a pragmatic One Health approach with robust collaboration among scientists, physicians, veterinarians, public health agencies, and industry stakeholders to develop and implement successful intervention strategies at both pre- and postharvest levels to effectively control UTIs caused by *E. coli* originating from poultry meat.

References

Achtman, M., & Pluschke, G. (1986). Clonal analysis of descent and virulence among selected Escherichia coli. *Annual Review of Microbiology, 40*, 185–210. https://doi.org/10.1146/annurev.mi.40.100186.001153

Adeyanju, G. T., & Ishola, O. (2014). Salmonella and Escherichia coli contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. *Springerplus, 3*, 139. https://doi.org/10.1186/2193-1801-3-139

Aliyu, A. B., Saleha, A. A., Jalila, A., & Zunita, Z. (2016). Risk factors and spatial distribution of extended spectrum beta-lactamase-producing-Escherichia coli at retail poultry meat markets in Malaysia: A cross-sectional study. *BMC Public Health, 16*, 699. https://doi.org/10.1186/s12889-016-3377-2

Allocati, N., Masulli, M., Alexeyev, M. F., & Di Ilio, C. (2013). Escherichia coli in Europe: An overview. *International Journal of Environmental Research and Public Health, 10*(12), 6235–6254. https://doi.org/10.3390/ijerph10126235

Bagshaw, S. M., & Laupland, K. B. (2006). Epidemiology of intensive care unit-acquired urinary tract infections. *Current Opinion in Infectious Diseases, 19*(1), 67–71.

Ballmer, K., Korczak, B. M., Kuhnert, P., Slickers, P., Ehricht, R., & Hachler, H. (2007). Fast DNA serotyping of Escherichia coli by use of an oligonucleotide microarray. *Journal of Clinical Microbiology, 45*(2), 370–379. https://doi.org/10.1128/jcm.01361-06

Banerjee, R., & Johnson, J. R. (2014). A new clone sweeps clean: The enigmatic emergence of Escherichia coli sequence type 131. *Antimicrobial Agents and Chemotherapy, 58*(9), 4997–5004. https://doi.org/10.1128/aac.02824-14

Banerjee, R., Robicsek, A., Kuskowski, M. A., Porter, S., Johnston, B. D., Sokurenko, E., et al. (2013). Molecular epidemiology of Escherichia coli sequence type 131 and its H30 and H30-Rx subclones among extended-spectrum-beta-lactamase-positive and -negative E. coli clinical isolates from the Chicago region, 2007 to 2010. *Antimicrobial Agents and Chemotherapy, 57*(12), 6385–6388. https://doi.org/10.1128/aac.01604-13

Barbieri, N. L., de Oliveira, A. L., Tejkowski, T. M., Pavanelo, D. B., Matter, L. B., Pinheiro, S. R., et al. (2015). Molecular characterization and clonal relationships among Escherichia coli strains isolated from broiler chickens with colisepticemia. *Foodborne Pathogens and Disease, 12*(1), 74–83. https://doi.org/10.1089/fpd.2014.1815

Bauchart, P., Germon, P., Bree, A., Oswald, E., Hacker, J., & Dobrindt, U. (2010). Pathogenomic comparison of human extraintestinal and avian pathogenic Escherichia coli--search for factors involved in host specificity or zoonotic potential. *Microbial Pathogenesis, 49*(3), 105–115. https://doi.org/10.1016/j.micpath.2010.05.004

Belanger, L., Garenaux, A., Harel, J., Boulianne, M., Nadeau, E., & Dozois, C. M. (2011). Escherichia coli from animal reservoirs as a potential source of human extraintestinal pathogenic E. coli. *FEMS Immunology and Medical Microbiology, 62*(1), 1–10. https://doi.org/10.1111/j.1574-695X.2011.00797.x

Berg, E. S., Wester, A. L., Ahrenfeldt, J., Mo, S. S., Slettemeas, J. S., Steinbakk, M., et al. (2017). Norwegian patients and retail chicken meat share cephalosporin-resistant Escherichia coli and IncK/blaCMY-2 resistance plasmids. *Clinical Microbiology and Infection, 23*(6), 407.e409–407.e415. https://doi.org/10.1016/j.cmi.2016.12.035

Bettelheim, K. A., Cooke, E. M., O'Farrell, S., & Shooter, R. A. (1977). The effect of diet on intestinal Escherichia coli. *The Journal of Hygiene, 79*(1), 43–45.

Bidet, P., Metais, A., Mahjoub-Messai, F., Durand, L., Dehem, M., Aujard, Y., et al. (2007). Detection and identification by PCR of a highly virulent phylogenetic subgroup among extraintestinal pathogenic Escherichia coli B2 strains. *Applied and Environmental Microbiology, 73*(7), 2373–2377. https://doi.org/10.1128/aem.02341-06

Bonacorsi, S., Clermont, O., Houdouin, V., Cordevant, C., Brahimi, N., Marecat, A., et al. (2003). Molecular analysis and experimental virulence of French and North American Escherichia coli neonatal meningitis isolates: Identification of a new virulent clone. *The Journal of Infectious Diseases, 187*(12), 1895–1906. https://doi.org/10.1086/375347

Bonkat, G., Muller, G., Braissant, O., Frei, R., Tschudin-Suter, S., Rieken, M., et al. (2013). Increasing prevalence of ciprofloxacin resistance in extended-spectrum-beta-lactamase-producing Escherichia coli urinary isolates. *World Journal of Urology, 31*(6), 1427–1432. https://doi.org/10.1007/s00345-013-1031-5

Bonnet, C., Diarrassouba, F., Brousseau, R., Masson, L., Topp, E., & Diarra, M. S. (2009). Pathotype and antibiotic resistance gene distributions of Escherichia coli isolates from broiler chickens raised on antimicrobial-supplemented diets. *Applied and Environmental Microbiology, 75*(22), 6955–6962. https://doi.org/10.1128/aem.00375-09

Brzuszkiewicz, E., Gottschalk, G., Ron, E., Hacker, J., & Dobrindt, U. (2009). Adaptation of Pathogenic E. coli to various niches: Genome flexibility is the key. *Genome Dynamics, 6*, 110–125. https://doi.org/10.1159/000235766

Burman, W. J., Breese, P. E., Murray, B. E., Singh, K. V., Batal, H. A., MacKenzie, T. D., et al. (2003). Conventional and molecular epidemiology of trimethoprim-sulfamethoxazole resistance among urinary Escherichia coli isolates. *The American Journal of Medicine, 115*(5), 358–364.

Chen, S. L., Wu, M., Henderson, J. P., Hooton, T. M., Hibbing, M. E., Hultgren, S. J., et al. (2013). Genomic diversity and fitness of E. coli strains recovered from the intestinal and urinary tracts of women with recurrent urinary tract infection. *Science Translational Medicine, 5*(184), 184ra160. https://doi.org/10.1126/scitranslmed.3005497

Clermont, O., Bonacorsi, S., & Bingen, E. (2000). Rapid and simple determination of the Escherichia coli phylogenetic group. *Applied and Environmental Microbiology, 66*(10), 4555–4558.

Clermont, O., Christenson, J. K., Denamur, E., & Gordon, D. M. (2013). The Clermont Escherichia coli phylo-typing method revisited: Improvement of specificity and detection of new phylo-groups. *Environmental Microbiology Reports, 5*(1), 58–65. https://doi.org/10.1111/1758-2229.12019

Clermont, O., Gordon, D., & Denamur, E. (2015). Guide to the various phylogenetic classification schemes for Escherichia coli and the correspondence among schemes. *Microbiology (Reading, England), 161*(Pt 5), 980–988. https://doi.org/10.1099/mic.0.000063

Clermont, O., Lavollay, M., Vimont, S., Deschamps, C., Forestier, C., Branger, C., et al. (2008). The CTX-M-15-producing Escherichia coli diffusing clone belongs to a highly virulent B2 phylogenetic subgroup. *The Journal of Antimicrobial Chemotherapy, 61*(5), 1024–1028. https://doi.org/10.1093/jac/dkn084

Colpan, A., Johnston, B., Porter, S., Clabots, C., Anway, R., Thao, L., et al. (2013). Escherichia coli sequence type 131 (ST131) subclone H30 as an emergent multidrug-resistant pathogen among US veterans. *Clinical Infectious Diseases, 57*(9), 1256–1265. https://doi.org/10.1093/cid/cit503

Coque, T. M., Novais, A., Carattoli, A., Poirel, L., Pitout, J., Peixe, L., et al. (2008). Dissemination of clonally related Escherichia coli strains expressing extended-spectrum beta-lactamase CTX-M-15. *Emerging Infectious Diseases, 14*(2), 195–200. https://doi.org/10.3201/eid1402.070350

Cordoba, G., Holm, A., Hansen, F., Hammerum, A. M., & Bjerrum, L. (2017). Prevalence of antimicrobial resistant Escherichia coli from patients with suspected urinary tract infection in primary care, Denmark. *BMC Infectious Diseases, 17*(1), 670. https://doi.org/10.1186/s12879-017-2785-y

Corpet, D. E. (1988). Antibiotic resistance from food. *The New England Journal of Medicine, 318*(18), 1206–1207.

Cortes, P., Blanc, V., Mora, A., Dahbi, G., Blanco, J. E., Blanco, M., et al. (2010). Isolation and characterization of potentially pathogenic antimicrobial-resistant Escherichia coli strains from chicken and pig farms in Spain. *Applied and Environmental Microbiology, 76*(9), 2799–2805. https://doi.org/10.1128/aem.02421-09

Courpon-Claudinon, A., Lefort, A., Panhard, X., Clermont, O., Dornic, Q., Fantin, B., et al. (2011). Bacteraemia caused by third-generation cephalosporin-resistant Escherichia coli in France: Prevalence, molecular epidemiology and clinical features. *Clinical Microbiology and Infection, 17*(4), 557–565. https://doi.org/10.1111/j.1469-0691.2010.03298.x

Crossman, L. C., Chaudhuri, R. R., Beatson, S. A., Wells, T. J., Desvaux, M., Cunningham, A. F., et al. (2010). A commensal gone bad: Complete genome sequence of the prototypical enterotoxigenic Escherichia coli strain H10407. *Journal of Bacteriology, 192*(21), 5822–5831. https://doi.org/10.1128/jb.00710-10

Croxen, M. A., & Finlay, B. B. (2010). Molecular mechanisms of Escherichia coli pathogenicity. *Nature Reviews Microbiology, 8*(1), 26–38. https://doi.org/10.1038/nrmicro2265

Dale, A. P., & Woodford, N. (2015). Extra-intestinal pathogenic Escherichia coli (ExPEC): Disease, carriage and clones. *The Journal of Infection, 71*(6), 615–626. https://doi.org/10.1016/j.jinf.2015.09.009

Dalmau, D., Navarro, F., Mirelis, B., Blanco, J., Garau, J., & Prats, G. (1996). Escherichia coli bacteraemia. Serotype O15:K52:H1 as a urinary pathogen. *The Journal of Hospital Infection, 34*(3), 233–234.

Danzeisen, J. L., Wannemuehler, Y., Nolan, L. K., & Johnson, T. J. (2013). Comparison of multilocus sequence analysis and virulence genotyping of Escherichia coli from live birds, retail poultry meat, and human extraintestinal infection. *Avian Diseases, 57*(1), 104–108. https://doi.org/10.1637/10218-042812-ResNote.1

DeFrances, C. J., Lucas, C. A., Buie, V. C., & Golosinskiy, A. (2008). 2006 National Hospital Discharge Survey. *National Health Statistics Reports, 5*, 1–20.

Dho-Moulin, M., & Fairbrother, J. M. (1999). Avian pathogenic Escherichia coli (APEC). *Veterinary Research, 30*(2–3), 299–316.

Dobrindt, U. (2005). (Patho-)genomics of Escherichia coli. *International Journal of Medical Microbiology, 295*(6–7), 357–371. https://doi.org/10.1016/j.ijmm.2005.07.009

Donnenberg, M. S., & Welch, R. (1996). Virulence determinants of uropathogenic Escherichia coli. In H. L. T. Mobley & J. W. Warren (Eds.), *Urinary tract infections: Molecular pathogenesis and clinical management* (pp. 135–174). Washington, D.C.: American Society of Microbiology.

Dziva, F., & Stevens, M. P. (2008). Colibacillosis in poultry: Unravelling the molecular basis of virulence of avian pathogenic Escherichia coli in their natural hosts. *Avian Pathology: Journal of the WVPA, 37*(4), 355–366. https://doi.org/10.1080/03079450802216652

Egea, P., Lopez-Cerero, L., Torres, E., Gomez-Sanchez Mdel, C., Serrano, L., Navarro Sanchez-Ortiz, M. D., et al. (2012). Increased raw poultry meat colonization by extended spectrum beta-lactamase-producing Escherichia coli in the south of Spain. *International Journal of Food Microbiology, 159*(2), 69–73. https://doi.org/10.1016/j.ijfoodmicro.2012.08.002

Ejrnaes, K. (2011). Bacterial characteristics of importance for recurrent urinary tract infections caused by Escherichia coli. *Danish Medical Bulletin, 58*(4), B4187.

EUCAST. (2011). European food safety authority. Scientific opinion on the public health risks of bacterial strains producing extended-spectrum b-lactamases and/or AmpC b-lactamases in food and food-producing animals. *EFSA Journal, 9*, 2322. http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/2322.pdf

Ewers, C., Antao, E. M., Diehl, I., Philipp, H. C., & Wieler, L. H. (2009). Intestine and environment of the chicken as reservoirs for extraintestinal pathogenic Escherichia coli strains with zoonotic potential. *Applied and Environmental Microbiology, 75*(1), 184–192. https://doi.org/10.1128/aem.01324-08

Ewers, C., Bethe, A., Semmler, T., Guenther, S., & Wieler, L. H. (2012). Extended-spectrum beta-lactamase-producing and AmpC-producing Escherichia coli from livestock and companion animals, and their putative impact on public health: A global perspective. *Clinical Microbiology and Infectious Diseases, 18*(7), 646–655. https://doi.org/10.1111/j.1469-0691.2012.03850.x

Ewers, C., Li, G., Wilking, H., Kiessling, S., Alt, K., Antao, E. M., et al. (2007). Avian pathogenic, uropathogenic, and newborn meningitis-causing Escherichia coli: How closely related are they? *International Journal of Medical Microbiology, 297*(3), 163–176. https://doi.org/10.1016/j.ijmm.2007.01.003

Fernandez-Alarcon, C., Singer, R. S., & Johnson, T. J. (2011). Comparative genomics of multidrug resistance-encoding IncA/C plasmids from commensal and pathogenic Escherichia coli from multiple animal sources. *PLoS One, 6*(8), e23415. https://doi.org/10.1371/journal.pone.0023415

Flores-Mireles, A. L., Walker, J. N., Caparon, M., & Hultgren, S. J. (2015). Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. *Nature Reviews Microbiology, 13*(5), 269–284. https://doi.org/10.1038/nrmicro3432

Foxman, B. (2002). Epidemiology of urinary tract infections: Incidence, morbidity, and economic costs. *The American Journal of Medicine, 113*(Suppl 1A), 5S–13S.

Foxman, B. (2003). Epidemiology of urinary tract infections: Incidence, morbidity, and economic costs. *Disease-a-Month: DM, 49*(2), 53–70. https://doi.org/10.1067/mda.2003.7

Foxman, B. (2010). The epidemiology of urinary tract infection. *Nature Reviews Urology, 7*(12), 653–660. https://doi.org/10.1038/nrurol.2010.190

Foxman, B. (2014). Urinary tract infection syndromes: Occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infectious Disease Clinics of North America, 28*(1), 1–13. https://doi.org/10.1016/j.idc.2013.09.003

Foxman, B., & Brown, P. (2003). Epidemiology of urinary tract infections: Transmission and risk factors, incidence, and costs. *Infectious disease clinics of North America, 17*(2), 227–241.

Foxman, B., Manning, S. D., Tallman, P., Bauer, R., Zhang, L., Koopman, J. S., et al. (2002). Uropathogenic Escherichia coli are more likely than commensal E. coli to be shared between heterosexual sex partners. *American Journal of Epidemiology, 156*(12), 1133–1140.

Foxman, B., Zhang, L., Tallman, P., Andree, B. C., Geiger, A. M., Koopman, J. S., et al. (1997). Transmission of uropathogens between sex partners. *The Journal of Infectious Diseases, 175*(4), 989–992.

Fratamico, P. M., DebRoy, C., Liu, Y., Needleman, D. S., Baranzoni, G. M., & Feng, P. (2016). Advances in molecular serotyping and subtyping of Escherichia coli. *Frontiers in Microbiology, 7*, 644. https://doi.org/10.3389/fmicb.2016.00644

Geerdes, H. F., Ziegler, D., Lode, H., Hund, M., Loehr, A., Fangmann, W., et al. (1992). Septicemia in 980 patients at a university hospital in Berlin: Prospective studies during 4 selected years between 1979 and 1989. *Clinical Infectious Diseases, 15*(6), 991–1002.

Ghodousi, A., Bonura, C., Di Carlo, P., van Leeuwen, W. B., & Mammina, C. (2016). Extraintestinal pathogenic Escherichia coli sequence type 131 H30-R and H30-Rx subclones in retail chicken meat, Italy. *International Journal of Food Microbiology, 228*, 10–13. https://doi.org/10.1016/j.ijfoodmicro.2016.04.004

Ghodousi, A., Bonura, C., Di Noto, A. M., & Mammina, C. (2015). Extended-spectrum beta-lactamase, AmpC-producing, and fluoroquinolone-resistant Escherichia coli in retail

broiler chicken meat, Italy. *Foodborne Pathogens and Disease, 12*(7), 619–625. https://doi.org/10.1089/fpd.2015.1936

Giufre, M., Graziani, C., Accogli, M., Luzzi, I., Busani, L., & Cerquetti, M. (2012). Escherichia coli of human and avian origin: Detection of clonal groups associated with fluoroquinolone and multidrug resistance in Italy. *The Journal of Antimicrobial Chemotherapy, 67*(4), 860–867. https://doi.org/10.1093/jac/dkr565

Gomi, R., Matsuda, T., Matsumura, Y., Yamamoto, M., Tanaka, M., Ichiyama, S., et al. (2017). Whole-genome analysis of antimicrobial-resistant and extraintestinal pathogenic Escherichia coli in river water. *Applied and Environmental Microbiology, 83*(5), e02703-16. https://doi.org/10.1128/aem.02703-16

Gordon, D. M., Clermont, O., Tolley, H., & Denamur, E. (2008). Assigning Escherichia coli strains to phylogenetic groups: Multi-locus sequence typing versus the PCR triplex method. *Environmental Microbiology, 10*(10), 2484–2496. https://doi.org/10.1111/j.1462-2920.2008.01669.x

Goullet, P., & Picard, B. (1989). Comparative electrophoretic polymorphism of esterases and other enzymes in Escherichia coli. *Journal of General Microbiology, 135*(1), 135–143. https://doi.org/10.1099/00221287-135-1-135

Gransden, W. R., Eykyn, S. J., Phillips, I., & Rowe, B. (1990). Bacteremia due to Escherichia coli: A study of 861 episodes. *Reviews of Infectious Diseases, 12*(6), 1008–1018.

Gruneberg, R. N. (1969). Relationship of infecting urinary organism to the faecal flora in patients with symptomatic urinary infection. *Lancet (London, England), 2*(7624), 766–768.

Gupta, K., Hooton, T. M., Naber, K. G., Wullt, B., Colgan, R., Miller, L. G., et al. (2011). International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clinical Infectious Diseases, 52*(5), e103–e120. https://doi.org/10.1093/cid/ciq257

Gupta, K., Sahm, D. F., Mayfield, D., & Stamm, W. E. (2001). Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in women: A nationwide analysis. *Clinical Infectious Diseases, 33*(1), 89–94. https://doi.org/10.1086/320880

Hadifar, S., Moghoofei, M., Nematollahi, S., Ramazanzadeh, R., Sedighi, M., Salehi-Abargouei, A., et al. (2017). Epidemiology of multidrug resistant uropathogenic Escherichia coli in Iran: A systematic review and meta-analysis. *Japanese Journal of Infectious Diseases, 70*(1), 19–25. https://doi.org/10.7883/yoken.JJID.2015.652

Hansen, K. H., Bortolaia, V., Nielsen, C. A., Nielsen, J. B., Schonning, K., Agerso, Y., et al. (2016). Host-specific patterns of genetic diversity among IncI1-Igamma and IncK plasmids encoding CMY-2 beta-lactamase in Escherichia coli isolates from humans, poultry meat, poultry, and dogs in Denmark. *Applied and Environmental Microbiology, 82*(15), 4705–4714. https://doi.org/10.1128/aem.00495-16

Hasman, H., Hammerum, A. M., Hansen, F., Hendriksen, R. S., Olesen, B., Agerso, Y., et al. (2015). Detection of mcr-1 encoding plasmid-mediated colistin-resistant Escherichia coli isolates from human bloodstream infection and imported chicken meat, Denmark 2015. *Euro Surveillance, 20*(49). https://doi.org/10.2807/1560-7917.es.2015.20.49.30085

Hooton, T. M., & Stamm, W. E. (1997). Diagnosis and treatment of uncomplicated urinary tract infection. *Infectious Disease Clinics of North America, 11*(3), 551–581.

Hussain, A., Ewers, C., Nandanwar, N., Guenther, S., Jadhav, S., Wieler, L. H., et al. (2012). Multiresistant uropathogenic Escherichia coli from a region in India where urinary tract infections are endemic: Genotypic and phenotypic characteristics of sequence type 131 isolates of the CTX-M-15 extended-spectrum-beta-lactamase-producing lineage. *Antimicrobial Agents and Chemotherapy, 56*(12), 6358–6365. https://doi.org/10.1128/aac.01099-12

Ingle, D. J., Valcanis, M., Kuzevski, A., Tauschek, M., Inouye, M., Stinear, T., et al. (2016). In silico serotyping of E. coli from short read data identifies limited novel O-loci but extensive diversity of O:H serotype combinations within and between pathogenic lineages. *Microbial Genomics, 2*(7), e000064. https://doi.org/10.1099/mgen.0.000064

Jakobsen, L., Garneau, P., Bruant, G., Harel, J., Olsen, S. S., Porsbo, L. J., et al. (2012). Is Escherichia coli urinary tract infection a zoonosis? Proof of direct link with production animals

and meat. European journal of clinical microbiology & infectious diseases: Official publication of the European Society of Clinical. *Microbiology, 31*(6), 1121–1129. https://doi.org/10.1007/s10096-011-1417-5

Jakobsen, L., Garneau, P., Kurbasic, A., Bruant, G., Stegger, M., Harel, J., et al. (2011). Microarray-based detection of extended virulence and antimicrobial resistance gene profiles in phylogroup B2 Escherichia coli of human, meat and animal origin. *Journal of Medical Microbiology, 60*(Pt 10), 1502–1511. https://doi.org/10.1099/jmm.0.033993-0

Jakobsen, L., Hammerum, A. M., & Frimodt-Moller, N. (2010a). Detection of clonal group A Escherichia coli isolates from broiler chickens, broiler chicken meat, community-dwelling humans, and urinary tract infection (UTI) patients and their virulence in a mouse UTI model. *Applied and Environmental Microbiology, 76*(24), 8281–8284. https://doi.org/10.1128/aem.01874-10

Jakobsen, L., Hammerum, A. M., & Frimodt-Moller, N. (2010b). Virulence of Escherichia coli B2 isolates from meat and animals in a murine model of ascending urinary tract infection (UTI): Evidence that UTI is a zoonosis. *Journal of Clinical Microbiology, 48*(8), 2978–2980. https://doi.org/10.1128/jcm.00281-10

Jakobsen, L., Kurbasic, A., Skjot-Rasmussen, L., Ejrnaes, K., Porsbo, L. J., Pedersen, K., et al. (2010c). Escherichia coli isolates from broiler chicken meat, broiler chickens, pork, and pigs share phylogroups and antimicrobial resistance with community-dwelling humans and patients with urinary tract infection. *Foodborne Pathogens and Disease, 7*(5), 537–547. https://doi.org/10.1089/fpd.2009.0409

Jakobsen, L., Spangholm, D. J., Pedersen, K., Jensen, L. B., Emborg, H. D., Agerso, Y., et al. (2010d). Broiler chickens, broiler chicken meat, pigs and pork as sources of ExPEC related virulence genes and resistance in Escherichia coli isolates from community-dwelling humans and UTI patients. *International Journal of Food Microbiology, 142*(1–2), 264–272. https://doi.org/10.1016/j.ijfoodmicro.2010.06.025

Johnson, J. R. (1991). Virulence factors in Escherichia coli urinary tract infection. *Clinical Microbiology Reviews, 4*(1), 80–128.

Johnson, J. R., & Clabots, C. (2006). Sharing of virulent Escherichia coli clones among household members of a woman with acute cystitis. *Clinical Infectious Diseases, 43*(10), e101–e108. https://doi.org/10.1086/508541

Johnson, J. R., Delavari, P., O'Bryan, T. T., Smith, K. E., & Tatini, S. (2005a). Contamination of retail foods, particularly Turkey, from community markets (Minnesota, 1999-2000) with antimicrobial-resistant and extraintestinal pathogenic Escherichia coli. *Foodborne Pathogens and Disease, 2*(1), 38–49. https://doi.org/10.1089/fpd.2005.2.38

Johnson, J. R., Johnston, B., Clabots, C., Kuskowski, M. A., & Castanheira, M. (2010). Escherichia coli sequence type ST131 as the major cause of serious multidrug-resistant E. coli infections in the United States. *Clinical Infectious Diseases, 51*(3), 286–294. https://doi.org/10.1086/653932

Johnson, J. R., Johnston, B., Kuskowski, M. A., Sokurenko, E. V., & Tchesnokova, V. (2015). Intensity and mechanisms of fluoroquinolone resistance within the H30 and H30Rx subclones of Escherichia coli sequence type 131 compared with other fluoroquinolone-resistant E. coli. *Antimicrobial Agents and Chemotherapy, 59*(8), 4471–4480. https://doi.org/10.1128/aac.00673-15

Johnson, J. R., Kaster, N., Kuskowski, M. A., & Ling, G. V. (2003). Identification of urovirulence traits in Escherichia coli by comparison of urinary and rectal E. coli isolates from dogs with urinary tract infection. *Journal of Clinical Microbiology, 41*(1), 337–345.

Johnson, J. R., Kuskowski, M. A., Menard, M., Gajewski, A., Xercavins, M., & Garau, J. (2006a). Similarity between human and chicken Escherichia coli isolates in relation to ciprofloxacin resistance status. *The Journal of Infectious Diseases, 194*(1), 71–78. https://doi.org/10.1086/504921

Johnson, J. R., Kuskowski, M. A., Smith, K., O'Bryan, T. T., & Tatini, S. (2005b). Antimicrobial-resistant and extraintestinal pathogenic Escherichia coli in retail foods. *The Journal of Infectious Diseases, 191*(7), 1040–1049. https://doi.org/10.1086/428451

Johnson, J. R., Manges, A. R., O'Bryan, T. T., & Riley, L. W. (2002). A disseminated multidrug-resistant clonal group of uropathogenic Escherichia coli in pyelonephritis. *Lancet (London, England), 359*(9325), 2249–2251. https://doi.org/10.1016/s0140-6736(02)09264-4

Johnson, J. R., Porter, S. B., Johnston, B., Thuras, P., Clock, S., Crupain, M., et al. (2017). Extraintestinal pathogenic and antimicrobial-resistant Escherichia coli, including sequence type 131 (ST131), from retail chicken breasts in the United States in 2013. *Applied and Environmental Microbiology, 83*(6), e02956-16. https://doi.org/10.1128/aem.02956-16

Johnson, J. R., & Russo, T. A. (2002). Extraintestinal pathogenic Escherichia coli: "the other bad E coli". *The Journal of Laboratory and Clinical Medicine, 139*(3), 155–162.

Johnson, J. R., Sannes, M. R., Croy, C., Johnston, B., Clabots, C., Kuskowski, M. A., et al. (2007a). Antimicrobial drug-resistant Escherichia coli from humans and poultry products, Minnesota and Wisconsin, 2002–2004. *Emerging Infectious Diseases, 13*(6), 838–846. https://doi.org/10.3201/eid1306.061576

Johnson, J. R., & Stamm, W. E. (1989). Urinary tract infections in women: Diagnosis and treatment. *Annals of Internal Medicine, 111*(11), 906–917.

Johnson, J. R., Stell, A. L., Delavari, P., Murray, A. C., Kuskowski, M., & Gaastra, W. (2001). Phylogenetic and pathotypic similarities between Escherichia coli isolates from urinary tract infections in dogs and extraintestinal infections in humans. *The Journal of Infectious Diseases, 183*(6), 897–906. https://doi.org/10.1086/319263

Johnson, J. R., Urban, C., Weissman, S. J., Jorgensen, J. H., Lewis 2nd, J. S., Hansen, G., et al. (2012a). Molecular epidemiological analysis of Escherichia coli sequence type ST131 (O25:H4) and blaCTX-M-15 among extended-spectrum-beta-lactamase-producing E. coli from the United States, 2000 to 2009. *Antimicrobial Agents and Chemotherapy, 56*(5), 2364–2370. https://doi.org/10.1128/aac.05824-11

Johnson, T. J., Johnson, S. J., & Nolan, L. K. (2006b). Complete DNA sequence of a ColBM plasmid from avian pathogenic Escherichia coli suggests that it evolved from closely related ColV virulence plasmids. *Journal of Bacteriology, 188*(16), 5975–5983. https://doi.org/10.1128/jb.00204-06

Johnson, T. J., Logue, C. M., Johnson, J. R., Kuskowski, M. A., Sherwood, J. S., Barnes, H. J., et al. (2012b). Associations between multidrug resistance, plasmid content, and virulence potential among extraintestinal pathogenic and commensal Escherichia coli from humans and poultry. *Foodborne Pathogens and Disease, 9*(1), 37–46. https://doi.org/10.1089/fpd.2011.0961

Johnson, T. J., Logue, C. M., Wannemuehler, Y., Kariyawasam, S., Doetkott, C., DebRoy, C., et al. (2009). Examination of the source and extended virulence genotypes of Escherichia coli contaminating retail poultry meat. *Foodborne Pathogens and Disease, 6*(6), 657–667. https://doi.org/10.1089/fpd.2009.0266

Johnson, T. J., Siek, K. E., Johnson, S. J., & Nolan, L. K. (2005c). DNA sequence and comparative genomics of pAPEC-O2-R, an avian pathogenic Escherichia coli transmissible R plasmid. *Antimicrobial Agents and Chemotherapy, 49*(11), 4681–4688. https://doi.org/10.1128/aac.49.11.4681-4688.2005

Johnson, T. J., Siek, K. E., Johnson, S. J., & Nolan, L. K. (2006c). DNA sequence of a ColV plasmid and prevalence of selected plasmid-encoded virulence genes among avian Escherichia coli strains. *Journal of Bacteriology, 188*(2), 745–758. https://doi.org/10.1128/jb.188.2.745-758.2006

Johnson, T. J., Skyberg, J., & Nolan, L. K. (2004). Multiple antimicrobial resistance region of a putative virulence plasmid from an Escherichia coli isolate incriminated in avian colibacillosis. *Avian Diseases, 48*(2), 351–360. https://doi.org/10.1637/7121

Johnson, T. J., Wannemeuhler, Y. M., Scaccianoce, J. A., Johnson, S. J., & Nolan, L. K. (2006d). Complete DNA sequence, comparative genomics, and prevalence of an IncHI2 plasmid occurring among extraintestinal pathogenic Escherichia coli isolates. *Antimicrobial Agents and Chemotherapy, 50*(11), 3929–3933. https://doi.org/10.1128/aac.00569-06

Johnson, T. J., Wannemuehler, Y., Doetkott, C., Johnson, S. J., Rosenberger, S. C., & Nolan, L. K. (2008a). Identification of minimal predictors of avian pathogenic Escherichia coli virulence for use as a rapid diagnostic tool. *Journal of Clinical Microbiology, 46*(12), 3987–3996. https://doi.org/10.1128/jcm.00816-08

Johnson, T. J., Wannemuehler, Y., Johnson, S. J., Stell, A. L., Doetkott, C., Johnson, J. R., et al. (2008b). Comparison of extraintestinal pathogenic Escherichia coli strains from human and avian sources reveals a mixed subset representing potential zoonotic pathogens. *Applied and Environmental Microbiology, 74*(22), 7043–7050. https://doi.org/10.1128/aem.01395-08

Johnson, T. J., Wannemuehler, Y. M., Johnson, S. J., Logue, C. M., White, D. G., Doetkott, C., et al. (2007b). Plasmid replicon typing of commensal and pathogenic Escherichia coli isolates. *Applied and Environmental Microbiology, 73*(6), 1976–1983. https://doi.org/10.1128/aem.02171-06

Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Pathogenic Escherichia coli. *Nature Reviews Microbiology, 2*(2), 123–140. https://doi.org/10.1038/nrmicro818

Karfunkel, D., Carmeli, Y., Chmelnitsky, I., Kotlovsky, T., & Navon-Venezia, S. (2013). The emergence and dissemination of CTX-M-producing Escherichia coli sequence type 131 causing community-onset bacteremia in Israel. *European Journal of Clinical Microbiology and Infectious Diseases, 32*(4), 513–521. https://doi.org/10.1007/s10096-012-1765-9

Kariyawasam, S., Scaccianoce, J. A., & Nolan, L. K. (2007). Common and specific genomic sequences of avian and human extraintestinal pathogenic Escherichia coli as determined by genomic subtractive hybridization. *BMC Microbiology, 7*, 81. https://doi.org/10.1186/1471-2180-7-81

Kauffmann, F. (1947). The serology of the coli group. *Journal of Immunology (Baltimore, MD: 1950), 57*(1), 71–100.

Kluytmans, J. A., Overdevest, I. T., Willemsen, I., Kluytmans-van den Bergh, M. F., van der Zwaluw, K., Heck, M., et al. (2013). Extended-spectrum beta-lactamase-producing Escherichia coli from retail chicken meat and humans: Comparison of strains, plasmids, resistance genes, and virulence factors. *Clinical Infectious Diseases, 56*(4), 478–487. https://doi.org/10.1093/cid/cis929

Kluytmans-van den Bergh, M. F., Huizinga, P., Bonten, M. J., Bos, M., De Bruyne, K., Friedrich, A. W., et al. (2016). Presence of mcr-1-positive Enterobacteriaceae in retail chicken meat but not in humans in the Netherlands since 2009. *Euro Surveillance, 21*(9), 30149. https://doi.org/10.2807/1560-7917.es.2016.21.9.30149

Kobayashi, R. K., Aquino, I., Ferreira, A. L., & Vidotto, M. C. (2011). EcoR phylogenetic analysis and virulence genotyping of avian pathogenic Escherichia coli strains and Escherichia coli isolates from commercial chicken carcasses in southern Brazil. *Foodborne Pathogens and Disease, 8*(5), 631–634. https://doi.org/10.1089/fpd.2010.0726

Koga, V. L., Rodrigues, G. R., Scandorieiro, S., Vespero, E. C., Oba, A., de Brito, B. G., et al. (2015). Evaluation of the antibiotic resistance and virulence of Escherichia coli strains isolated from chicken carcasses in 2007 and 2013 from Parana, Brazil. *Foodborne Pathogens and Disease, 12*(6), 479–485. https://doi.org/10.1089/fpd.2014.1888

Kohler, C. D., & Dobrindt, U. (2011). What defines extraintestinal pathogenic Escherichia coli? *International Journal of Medical Microbiology, 301*(8), 642–647. https://doi.org/10.1016/j.ijmm.2011.09.006

Kucheria, R., Dasgupta, P., Sacks, S. H., Khan, M. S., & Sheerin, N. S. (2005). Urinary tract infections: New insights into a common problem. *Postgraduate Medical Journal, 81*(952), 83–86. https://doi.org/10.1136/pgmj.2004.023036

Le, Q. P., Ueda, S., Nguyen, T. N., Dao, T. V., Van Hoang, T. A., Tran, T. T., et al. (2015). Characteristics of extended-spectrum beta-lactamase-producing Escherichia coli in retail meats and shrimp at a local market in Vietnam. *Foodborne Pathogens and Disease, 12*(8), 719–725. https://doi.org/10.1089/fpd.2015.1954

Leimbach, A., Hacker, J., & Dobrindt, U. (2013). E. coli as an all-rounder: The thin line between commensalism and pathogenicity. *Current Topics in Microbiology and Immunology, 358*, 3–32. https://doi.org/10.1007/82_2012_303

Levy, S. B., FitzGerald, G. B., & Macone, A. B. (1976). Spread of antibiotic-resistant plasmids from chicken to chicken and from chicken to man. *Nature, 260*(5546), 40–42.

Lim, E. J., Ho, S. X., Cao, D. Y., Lau, Q. C., Koh, T. H., & Hsu, L. Y. (2016). Extended-spectrum beta-lactamase-producing Enterobacteriaceae in retail chicken meat in Singapore. *Annals of the Academy of Medicine, Singapore, 45*(12), 557–559.

Lima-Filho, J. V., Martins, L. V., Nascimento, D. C., Ventura, R. F., Batista, J. E., Silva, A. F., et al. (2013). Zoonotic potential of multidrug-resistant extraintestinal pathogenic Escherichia coli obtained from healthy poultry carcasses in Salvador, Brazil. *The Brazilian Journal of Infectious Diseases, 17*(1), 54–61. https://doi.org/10.1016/j.bjid.2012.09.004

Literak, I., Reitschmied, T., Bujnakova, D., Dolejska, M., Cizek, A., Bardon, J., et al. (2013). Broilers as a source of quinolone-resistant and extraintestinal pathogenic Escherichia coli in the Czech Republic. *Microbial Drug Resistance (Larchmont, NY), 19*(1), 57–63. https://doi.org/10.1089/mdr.2012.0124

Litwin, M. S., Saigal, C. S., Yano, E. M., Avila, C., Geschwind, S. A., Hanley, J. M., et al. (2005). Urologic diseases in America project: Analytical methods and principal findings. *The Journal of Urology, 173*(3), 933–937. https://doi.org/10.1097/01.ju.0000152365.43125.3b

Liu, Y. Y., Wang, Y., Walsh, T. R., Yi, L. X., Zhang, R., Spencer, J., et al. (2016). Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: A microbiological and molecular biological study. *The Lancet Infectious Diseases, 16*(2), 161–168. https://doi.org/10.1016/s1473-3099(15)00424-7

Logue, C. M., Doetkott, C., Mangiamele, P., Wannemuehler, Y. M., Johnson, T. J., Tivendale, K. A., et al. (2012). Genotypic and phenotypic traits that distinguish neonatal meningitis-associated Escherichia coli from fecal E. coli isolates of healthy human hosts. *Applied and Environmental Microbiology, 78*(16), 5824–5830. https://doi.org/10.1128/aem.07869-11

Logue, C. M., Wannemuehler, Y., Nicholson, B. A., Doetkott, C., Barbieri, N. L., & Nolan, L. K. (2017). Comparative analysis of phylogenetic assignment of human and avian ExPEC and Fecal commensal Escherichia coli using the (previous and revised) Clermont phylogenetic typing methods and its impact on avian pathogenic Escherichia coli (APEC) classification. *Frontiers in Microbiology, 8*, 283. https://doi.org/10.3389/fmicb.2017.00283

Lyhs, U., Ikonen, I., Pohjanvirta, T., Raninen, K., Perko-Makela, P., & Pelkonen, S. (2012). Extraintestinal pathogenic Escherichia coli in poultry meat products on the Finnish retail market. *Acta Veterinaria Scandinavica, 54*, 64. https://doi.org/10.1186/1751-0147-54-64

Maiden, M. C., Bygraves, J. A., Feil, E., Morelli, G., Russell, J. E., Urwin, R., et al. (1998). Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. *Proceedings of the National Academy of Sciences of the United States of America, 95*(6), 3140–3145.

Maluta, R. P., Logue, C. M., Casas, M. R., Meng, T., Guastalli, E. A., Rojas, T. C., et al. (2014). Overlapped sequence types (STs) and serogroups of avian pathogenic (APEC) and human extra-intestinal pathogenic (ExPEC) Escherichia coli isolated in Brazil. *PLoS One, 9*(8), e105016. https://doi.org/10.1371/journal.pone.0105016

Manges, A. R. (2016). Escherichia coli and urinary tract infections: The role of poultry-meat. *Clinical Microbiology and Infection, 22*(2), 122–129. https://doi.org/10.1016/j.cmi.2015.11.010

Manges, A. R., Johnson, J. R., Foxman, B., O'Bryan, T. T., Fullerton, K. E., & Riley, L. W. (2001). Widespread distribution of urinary tract infections caused by a multidrug-resistant Escherichia coli clonal group. *The New England Journal of Medicine, 345*(14), 1007–1013. https://doi.org/10.1056/NEJMoa011265

Manges, A. R., Mende, K., Murray, C. K., Johnston, B. D., Sokurenko, E. V., Tchesnokova, V., et al. (2017). Clonal distribution and associated characteristics of Escherichia coli clinical and surveillance isolates from a military medical center. *Diagnostic Microbiology and Infectious Disease, 87*(4), 382–385. https://doi.org/10.1016/j.diagmicrobio.2017.01.007

Manges, A. R., Smith, S. P., Lau, B. J., Nuval, C. J., Eisenberg, J. N., Dietrich, P. S., et al. (2007). Retail meat consumption and the acquisition of antimicrobial resistant Escherichia coli causing urinary tract infections: A case-control study. *Foodborne Pathogens and Disease, 4*(4), 419–431. https://doi.org/10.1089/fpd.2007.0026

Markland, S. M., LeStrange, K. J., Sharma, M., & Kniel, K. E. (2015). Old friends in new places: Exploring the role of extraintestinal E. coli in intestinal disease and foodborne illness. *Zoonoses and Public Health, 62*(7), 491–496. https://doi.org/10.1111/zph.12194

Marrs, C. F., Zhang, L., & Foxman, B. (2005). Escherichia coli mediated urinary tract infections: Are there distinct uropathogenic E. coli (UPEC) pathotypes? *FEMS Microbiology Letters, 252*(2), 183–190. https://doi.org/10.1016/j.femsle.2005.08.028

Mathers, A. J., Peirano, G., & Pitout, J. D. (2015). The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. *Clinical Microbiology Reviews, 28*(3), 565–591. https://doi.org/10.1128/cmr.00116-14

Maturana, V. G., de Pace, F., Carlos, C., Mistretta Pires, M., Amabile de Campos, T., Nakazato, G., et al. (2011). Subpathotypes of avian pathogenic Escherichia coli (APEC) exist as defined by their syndromes and virulence traits. *The Open Microbiology Journal, 5*(Suppl 1), 55–64. https://doi.org/10.2174/1874285801105010055

Mehnert-Kay, S. A. (2005). Diagnosis and management of uncomplicated urinary tract infections. *American Family Physician, 72*(3), 451–456.

Mellata, M. (2013). Human and avian extraintestinal pathogenic Escherichia coli: Infections, zoonotic risks, and antibiotic resistance trends. *Foodborne Pathogens and Disease, 10*(11), 916–932. https://doi.org/10.1089/fpd.2013.1533

Mellata, M., Ameiss, K., Mo, H., & Curtiss 3rd, R. (2010). Characterization of the contribution to virulence of three large plasmids of avian pathogenic Escherichia coli chi7122 (O78:K80:H9). *Infection and Immunity, 78*(4), 1528–1541. https://doi.org/10.1128/iai.00981-09

Mellata, M., Johnson, J. R., & Curtiss 3rd, R. (2018). Escherichia coli isolates from commercial chicken meat and eggs cause sepsis, meningitis and urinary tract infection in rodent models of human infections. *Zoonoses and Public Health, 65*(1), 103–113. https://doi.org/10.1111/zph.12376

Mellata, M., Maddux, J. T., Nam, T., Thomson, N., Hauser, H., Stevens, M. P., et al. (2012). New insights into the bacterial fitness-associated mechanisms revealed by the characterization of large plasmids of an avian pathogenic E. coli. *PLoS One, 7*(1), e29481. https://doi.org/10.1371/journal.pone.0029481

Mellata, M., Touchman, J. W., & Curtiss, R. (2009). Full sequence and comparative analysis of the plasmid pAPEC-1 of avian pathogenic E. coli chi7122 (O78:K80:H9). *PLoS One, 4*(1), e4232. https://doi.org/10.1371/journal.pone.0004232

Mitchell, N. M., Johnson, J. R., Johnston, B., Curtiss 3rd, R., & Mellata, M. (2015). Zoonotic potential of Escherichia coli isolates from retail chicken meat products and eggs. *Applied and Environmental Microbiology, 81*(3), 1177–1187. https://doi.org/10.1128/aem.03524-14

Mo, S. S., Slettemeas, J. S., Berg, E. S., Norstrom, M., & Sunde, M. (2016). Plasmid and host strain characteristics of Escherichia coli resistant to extended-spectrum cephalosporins in the Norwegian broiler production. *PLoS One, 11*(4), e0154019. https://doi.org/10.1371/journal.pone.0154019

Mora, A., Lopez, C., Herrera, A., Viso, S., Mamani, R., Dhabi, G., et al. (2012). Emerging avian pathogenic Escherichia coli strains belonging to clonal groups O111:H4-D-ST2085 and O111:H4-D-ST117 with high virulence-gene content and zoonotic potential. *Veterinary Microbiology, 156*(3–4), 347–352. https://doi.org/10.1016/j.vetmic.2011.10.033

Moreno, E., Andreu, A., Perez, T., Sabate, M., Johnson, J. R., & Prats, G. (2006). Relationship between Escherichia coli strains causing urinary tract infection in women and the dominant faecal flora of the same hosts. *Epidemiology and Infection, 134*(5), 1015–1023. https://doi.org/10.1017/s0950268806005917

Moulin-Schouleur, M., Reperant, M., Laurent, S., Bree, A., Mignon-Grasteau, S., Germon, P., et al. (2007). Extraintestinal pathogenic Escherichia coli strains of avian and human origin: Link between phylogenetic relationships and common virulence patterns. *Journal of Clinical Microbiology, 45*(10), 3366–3376. https://doi.org/10.1128/jcm.00037-07

Murray, A. C., Kuskowski, M. A., & Johnson, J. R. (2004). Virulence factors predict Escherichia coli colonization patterns among human and animal household members. *Annals of Internal Medicine, 140*(10), 848–849.

Nandanwar, N., Janssen, T., Kuhl, M., Ahmed, N., Ewers, C., & Wieler, L. H. (2014). Extraintestinal pathogenic Escherichia coli (ExPEC) of human and avian origin belonging to sequence type complex 95 (STC95) portray indistinguishable virulence features. *International Journal of Medical Microbiology, 304*(7), 835–842. https://doi.org/10.1016/j.ijmm.2014.06.009

Nicolas-Chanoine, M. H., Bertrand, X., & Madec, J. Y. (2014). Escherichia coli ST131, an intriguing clonal group. *Clinical Microbiology Reviews, 27*(3), 543–574. https://doi.org/10.1128/cmr.00125-13

Nicolas-Chanoine, M. H., Blanco, J., Leflon-Guibout, V., Demarty, R., Alonso, M. P., Canica, M. M., et al. (2008). Intercontinental emergence of Escherichia coli clone O25:H4-ST131 producing CTX-M-15. *The Journal of Antimicrobial Chemotherapy, 61*(2), 273–281. https://doi.org/10.1093/jac/dkm464

Nolan, L. K., Barnes, H. B., Vaillancourt, J. P., Abdul-Aziz, T., & Logue, C. M. (2003). Colibacillosis. In D. E. Swayne, J. R. Glisson, L. R. McDougald, L. K. Nolan, D. L. Suarez, & V. L. Nair (Eds.), *Diseases of poultry* (Vol. 13, pp. 631–652). Ames, IA: Iowa State University Press.

NORM/NORM-VET (2011). Usage of antimicrobial agents and occurrence of antimicrobial resistance in Norway. Tromsø/Oslo. 2012. ISSN:1502e2307 (print)/1890-9965 (electronic).

NORM/NORM-VET (2012). Usage of antimicrobial agents and occurrence of antimicrobial resistance in Norway. Tromsø/Oslo. 2013. ISSN:1502e2307 (print)/1890-9965 (electronic).

NORM/NORM-VET (2014). Usage of antimicrobial agents and occurrence of antimicrobial resistance in Norway. Tromsø/Oslo. 2015. ISSN:1502e2307 (print)/1890-9965 (electronic).

Obata-Yasuoka, M., Ba-Thein, W., Tsukamoto, T., Yoshikawa, H., & Hayashi, H. (2002). Vaginal Escherichia coli share common virulence factor profiles, serotypes and phylogeny with other extraintestinal E. coli. *Microbiology (Reading, England), 148*(Pt 9), 2745–2752. https://doi.org/10.1099/00221287-148-9-2745

Ochman, H., & Selander, R. K. (1984a). Evidence for clonal population structure in Escherichia coli. *Proceedings of the National Academy of Sciences of the United States of America, 81*(1), 198–201.

Ochman, H., & Selander, R. K. (1984b). Standard reference strains of Escherichia coli from natural populations. *Journal of Bacteriology, 157*(2), 690–693.

Olesen, B., Kolmos, H. J., Orskov, F., & Orskov, I. (1994). Cluster of multiresistant Escherichia coli O78:H10 in greater Copenhagen. *Scandinavian Journal of Infectious Diseases, 26*(4), 406–410.

Olsen, R. H., Christensen, H., & Bisgaard, M. (2012). Comparative genomics of multiple plasmids from APEC associated with clonal outbreaks demonstrates major similarities and identifies several potential vaccine-targets. *Veterinary Microbiology, 158*(3–4), 384–393. https://doi.org/10.1016/j.vetmic.2012.03.008

Orskov, I., Orskov, F., Jann, B., & Jann, K. (1977). Serology, chemistry, and genetics of O and K antigens of Escherichia coli. *Bacteriological Reviews, 41*(3), 667–710.

Orskov, I., Orskov, F., & Rowe, B. (1984). Six new E. coli O groups: O165, O166, O167, O168, O169 and O170. *Acta Pathologica, Microbiologica, et Immunologica Scandinavica. Section B, 92*(4), 189–193.

Ozawa, M., Baba, K., & Asai, T. (2010). Molecular typing of avian pathogenic Escherichia coli O78 strains in Japan by using multilocus sequence typing and pulsed-field gel electrophoresis. *The Journal of Veterinary Medical Science, 72*(11), 1517–1520.

Pehlivanlar Onen, S., Aslantas, O., Sebnem Yilmaz, E., & Kurekci, C. (2015). Prevalence of beta-lactamase producing Escherichia coli from retail meat in Turkey. *Journal of Food Science, 80*(9), M2023–M2029. https://doi.org/10.1111/1750-3841.12984

Peirano, G., Schreckenberger, P. C., & Pitout, J. D. (2011). Characteristics of NDM-1-producing Escherichia coli isolates that belong to the successful and virulent clone ST131. *Antimicrobial Agents and Chemotherapy, 55*(6), 2986–2988. https://doi.org/10.1128/aac.01763-10

Peirano, G., van der Bij, A. K., Gregson, D. B., & Pitout, J. D. (2012). Molecular epidemiology over an 11-year period (2000 to 2010) of extended-spectrum beta-lactamase-producing Escherichia coli causing bacteremia in a centralized Canadian region. *Journal of Clinical Microbiology, 50*(2), 294–299. https://doi.org/10.1128/jcm.06025-11

Petty, N. K., Ben Zakour, N. L., Stanton-Cook, M., Skippington, E., Totsika, M., Forde, B. M., et al. (2014). Global dissemination of a multidrug resistant Escherichia coli clone. *Proceedings of the National Academy of Sciences of the United States of America, 111*(15), 5694–5699. https://doi.org/10.1073/pnas.1322678111

Phillips, I., Eykyn, S., King, A., Gransden, W. R., Rowe, B., Frost, J. A., et al. (1988). Epidemic multiresistant Escherichia coli infection in West Lambeth Health District. *Lancet (London, England), 1*(8593), 1038–1041.

Picard, B., Garcia, J. S., Gouriou, S., Duriez, P., Brahimi, N., Bingen, E., et al. (1999). The link between phylogeny and virulence in Escherichia coli extraintestinal infection. *Infection and Immunity, 67*(2), 546–553.

Pitout, J. D. (2012). Extraintestinal pathogenic Escherichia coli: A combination of virulence with antibiotic resistance. *Frontiers in Microbiology, 3*, 9. https://doi.org/10.3389/fmicb.2012.00009

Pitout, J. D., Gregson, D. B., Church, D. L., Elsayed, S., & Laupland, K. B. (2005). Community-wide outbreaks of clonally related CTX-M-14 beta-lactamase-producing Escherichia coli strains in the Calgary health region. *Journal of Clinical Microbiology, 43*(6), 2844–2849. https://doi.org/10.1128/jcm.43.6.2844-2849.2005

Platell, J. L., Johnson, J. R., Cobbold, R. N., & Trott, D. J. (2011). Multidrug-resistant extraintestinal pathogenic Escherichia coli of sequence type ST131 in animals and foods. *Veterinary Microbiology, 153*(1–2), 99–108. https://doi.org/10.1016/j.vetmic.2011.05.007

Prats, G., Navarro, F., Mirelis, B., Dalmau, D., Margall, N., Coll, P., et al. (2000). Escherichia coli serotype O15:K52:H1 as a uropathogenic clone. *Journal of Clinical Microbiology, 38*(1), 201–209.

Price, L. B., Johnson, J. R., Aziz, M., Clabots, C., Johnston, B., Tchesnokova, V., et al. (2013). The epidemic of extended-spectrum-beta-lactamase-producing Escherichia coli ST131 is driven by a single highly pathogenic subclone, H30-Rx. *MBio, 4*(6), e00377–e00313. https://doi.org/10.1128/mBio.00377-13

Pupo, G. M., Karaolis, D. K., Lan, R., & Reeves, P. R. (1997). Evolutionary relationships among pathogenic and nonpathogenic Escherichia coli strains inferred from multilocus enzyme electrophoresis and mdh sequence studies. *Infection and Immunity, 65*(7), 2685–2692.

Ribot, E. M., Fair, M. A., Gautom, R., Cameron, D. N., Hunter, S. B., Swaminathan, B., et al. (2006). Standardization of pulsed-field gel electrophoresis protocols for the subtyping of Escherichia coli O157:H7, Salmonella, and Shigella for PulseNet. *Foodborne Pathogens and Disease, 3*(1), 59–67. https://doi.org/10.1089/fpd.2006.3.59

Riley, L. W. (2014). Pandemic lineages of extraintestinal pathogenic Escherichia coli. *Clinical Microbiology and Infection, 20*(5), 380–390. https://doi.org/10.1111/1469-0691.12646

Rodriguez-Siek, K. E., Giddings, C. W., Doetkott, C., Johnson, T. J., Fakhr, M. K., & Nolan, L. K. (2005a). Comparison of Escherichia coli isolates implicated in human urinary tract infection and avian colibacillosis. *Microbiology (Reading, England), 151*(Pt 6), 2097–2110. https://doi.org/10.1099/mic.0.27499-0

Rodriguez-Siek, K. E., Giddings, C. W., Doetkott, C., Johnson, T. J., & Nolan, L. K. (2005b). Characterizing the APEC pathotype. *Veterinary Research, 36*(2), 241–256. https://doi.org/10.1051/vetres:2004057

Ronco, T., Stegger, M., Olsen, R. H., Sekse, C., Nordstoga, A. B., Pohjanvirta, T., et al. (2017). Spread of avian pathogenic Escherichia coli ST117 O78:H4 in Nordic broiler production. *BMC Genomics, 18*(1), 13. https://doi.org/10.1186/s12864-016-3415-6

Russo, T. A., & Johnson, J. R. (2003). Medical and economic impact of extraintestinal infections due to Escherichia coli: Focus on an increasingly important endemic problem. *Microbes and Infection, 5*(5), 449–456.

Sannes, M. R., Kuskowski, M. A., Owens, K., Gajewski, A., & Johnson, J. R. (2004). Virulence factor profiles and phylogenetic background of Escherichia coli isolates from veterans with bacteremia and uninfected control subjects. *The Journal of Infectious Diseases, 190*(12), 2121–2128. https://doi.org/10.1086/425984

Scheutz, F., Cheasty, T., Woodward, D., & Smith, H. R. (2004). Designation of O174 and O175 to temporary O groups OX3 and OX7, and six new E. coli O groups that include Verocytotoxin-producing E. coli (VTEC): O176, O177, O178, O179, O180 and O181. *APMIS, 112*(9), 569–584. https://doi.org/10.1111/j.1600-0463.2004.apm1120903.x

Schito, G. C., Naber, K. G., Botto, H., Palou, J., Mazzei, T., Gualco, L., et al. (2009). The ARESC study: An international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infections. *International Journal of Antimicrobial Agents, 34*(5), 407–413. https://doi.org/10.1016/j.ijantimicag.2009.04.012

Selander, R. K., Caugant, D. A., Ochman, H., Musser, J. M., Gilmour, M. N., & Whittam, T. S. (1986). Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. *Applied and Environmental Microbiology, 51*(5), 873–884.

Sheikh, A. A., Checkley, S., Avery, B., Chalmers, G., Bohaychuk, V., Boerlin, P., et al. (2012). Antimicrobial resistance and resistance genes in Escherichia coli isolated from retail meat purchased in Alberta, Canada. *Foodborne Pathogens and Disease, 9*(7), 625–631. https://doi.org/10.1089/fpd.2011.1078

Silverman, J. A., Schreiber 4th, H. L., Hooton, T. M., & Hultgren, S. J. (2013). From physiology to pharmacy: Developments in the pathogenesis and treatment of recurrent urinary tract infections. *Current Urology Reports, 14*(5), 448–456. https://doi.org/10.1007/s11934-013-0354-5

Skyberg, J. A., Johnson, T. J., Johnson, J. R., Clabots, C., Logue, C. M., & Nolan, L. K. (2006). Acquisition of avian pathogenic Escherichia coli plasmids by a commensal E. coli isolate enhances its abilities to kill chicken embryos, grow in human urine, and colonize the murine kidney. *Infection and Immunity, 74*(11), 6287–6292. https://doi.org/10.1128/iai.00363-06

Skyberg, J. A., Johnson, T. J., & Nolan, L. K. (2008). Mutational and transcriptional analyses of an avian pathogenic Escherichia coli ColV plasmid. *BMC Microbiology, 8*, 24. https://doi.org/10.1186/1471-2180-8-24

Smith, J. L., Fratamico, P. M., & Gunther, N. W. (2007). Extraintestinal pathogenic Escherichia coli. *Foodborne Pathogens and Disease, 4*(2), 134–163. https://doi.org/10.1089/fpd.2007.0087

Sorsa, L. J., Dufke, S., Heesemann, J., & Schubert, S. (2003). Characterization of an iroBCDEN gene cluster on a transmissible plasmid of uropathogenic Escherichia coli: Evidence for horizontal transfer of a chromosomal virulence factor. *Infection and Immunity, 71*(6), 3285–3293.

Spurbeck, R. R., Dinh Jr., P. C., Walk, S. T., Stapleton, A. E., Hooton, T. M., Nolan, L. K., et al. (2012). Escherichia coli isolates that carry vat, fyuA, chuA, and yfcV efficiently colonize the urinary tract. *Infection and Immunity, 80*(12), 4115–4122. https://doi.org/10.1128/iai.00752-12

Stamm, W. E., & Hooton, T. M. (1993). Management of urinary tract infections in adults. *The New England Journal of Medicine, 329*(18), 1328–1334. https://doi.org/10.1056/nejm199310283291808

Stenutz, R., Weintraub, A., & Widmalm, G. (2006). The structures of Escherichia coli O-polysaccharide antigens. *FEMS Microbiology Reviews, 30*(3), 382–403. https://doi.org/10.1111/j.1574-6976.2006.00016.x

Stromberg, Z. R., Johnson, J. R., Fairbrother, J. M., Kilbourne, J., Van Goor, A., Curtiss, R. R., et al. (2017). Evaluation of Escherichia coli isolates from healthy chickens to determine their potential risk to poultry and human health. *PLoS One, 12*(7), e0180599. https://doi.org/10.1371/journal.pone.0180599

Terlizzi, M. E., Gribaudo, G., & Maffei, M. E. (2017). UroPathogenic Escherichia coli (UPEC) infections: Virulence factors, bladder responses, antibiotic, and non-antibiotic antimicrobial strategies. *Frontiers in Microbiology, 8*, 1566. https://doi.org/10.3389/fmicb.2017.01566

Tivendale, K. A., Allen, J. L., & Browning, G. F. (2009a). Plasmid-borne virulence-associated genes have a conserved organization in virulent strains of avian pathogenic Escherichia coli. *Journal of Clinical Microbiology, 47*(8), 2513–2519. https://doi.org/10.1128/jcm.00391-09

Tivendale, K. A., Noormohammadi, A. H., Allen, J. L., & Browning, G. F. (2009b). The conserved portion of the putative virulence region contributes to virulence of avian pathogenic Escherichia coli. *Microbiology (Reading, England), 155*(Pt 2), 450–460. https://doi.org/10.1099/mic.0.023143-0

Toth, I., Dobrindt, U., Koscso, B., Kosa, A., Herpay, M., & Nagy, B. (2012). Genetic and phylogenetic analysis of avian extraintestinal and intestinal Escherichia coli. *Acta Microbiologica et Immunologica Hungarica, 59*(3), 393–409. https://doi.org/10.1556/AMicr.59.2012.3.10

Touchon, M., Hoede, C., Tenaillon, O., Barbe, V., Baeriswyl, S., Bidet, P., et al. (2009). Organised genome dynamics in the Escherichia coli species results in highly diverse adaptive paths. *PLoS Genetics, 5*(1), e1000344. https://doi.org/10.1371/journal.pgen.1000344

Trampel, D. W., Wannemuehler, Y., & Nolan, L. K. (2007). Characterization of Escherichia coli isolates from peritonitis lesions in commercial laying hens. *Avian Diseases, 51*(4), 840–844. https://doi.org/10.1637/7797-111906-regr1.1

Vincent, C., Boerlin, P., Daignault, D., Dozois, C. M., Dutil, L., Galanakis, C., et al. (2010). Food reservoir for Escherichia coli causing urinary tract infections. *Emerging Infectious Diseases, 16*(1), 88–95. https://doi.org/10.3201/eid1601.091118

Voets, G. M., Fluit, A. C., Scharringa, J., Schapendonk, C., van den Munckhof, T., Leverstein-van Hall, M. A., et al. (2013). Identical plasmid AmpC beta-lactamase genes and plasmid types in E. coli isolates from patients and poultry meat in the Netherlands. *International Journal of Food Microbiology, 167*(3), 359–362. https://doi.org/10.1016/j.ijfoodmicro.2013.10.001

Warren, J. W. (1996). Clinical presentations and epidemiology of urinary tract infections. In L. T. Harry & J. Warren (Eds.), *Urinary tract infections: Molecular pathogenesis and clinical management*. Washington, D.C.: ASM Press.

Weissman, S. J., Johnson, J. R., Tchesnokova, V., Billig, M., Dykhuizen, D., Riddell, K., et al. (2012). High-resolution two-locus clonal typing of extraintestinal pathogenic Escherichia coli. *Applied and Environmental Microbiology, 78*(5), 1353–1360. https://doi.org/10.1128/aem.06663-11

WHO. (2014). *Antimicrobial resistance: global report on surveillance*. Retrieved October 25, 2017, from http://www.who.int/drugresistance/documents/surveillancereport/en/

Wijetunge, D. S., Gongati, S., DebRoy, C., Kim, K. S., Couraud, P. O., Romero, I. A., et al. (2015). Characterizing the pathotype of neonatal meningitis causing Escherichia coli (NMEC). *BMC Microbiology, 15*, 211. https://doi.org/10.1186/s12866-015-0547-9

Wirth, T., Falush, D., Lan, R., Colles, F., Mensa, P., Wieler, L. H., et al. (2006). Sex and virulence in Escherichia coli: An evolutionary perspective. *Molecular Microbiology, 60*(5), 1136–1151. https://doi.org/10.1111/j.1365-2958.2006.05172.x

Woodford, N., Kaufmann, M. E., Karisik, E., & Hartley, J. W. (2007). Molecular epidemiology of multiresistant Escherichia coli isolates from community-onset urinary tract infections in Cornwall, England. *The Journal of Antimicrobial Chemotherapy, 59*(1), 106–109. https://doi.org/10.1093/jac/dkl435

Wu, Q., Xi, M., Lv, X., Xu, Y., Feng, Y., Li, Q., et al. (2014). Presence and antimicrobial susceptibility of Escherichia coli recovered from retail chicken in China. *Journal of Food Protection, 77*(10), 1773–1777. https://doi.org/10.4315/0362-028x.jfp-14-080

Yamamoto, S., Tsukamoto, T., Terai, A., Kurazono, H., Takeda, Y., & Yoshida, O. (1997). Genetic evidence supporting the fecal-perineal-urethral hypothesis in cystitis caused by Escherichia coli. *The Journal of Urology, 157*(3), 1127–1129.

Zhanel, G. G., Hisanaga, T. L., Laing, N. M., DeCorby, M. R., Nichol, K. A., Palatnik, L. P., et al. (2005). Antibiotic resistance in outpatient urinary isolates: Final results from the North American urinary tract infection collaborative alliance (NAUTICA). *International Journal of Antimicrobial Agents, 26*(5), 380–388. https://doi.org/10.1016/j.ijantimicag.2005.08.003

Zhang, L., & Foxman, B. (2003). Molecular epidemiology of Escherichia coli mediated urinary tract infections. *Frontiers in Bioscience: a Journal and Virtual Library, 8*, e235–e244.

Zhang, L., Foxman, B., & Marrs, C. (2002). Both urinary and rectal Escherichia coli isolates are dominated by strains of phylogenetic group B2. *Journal of Clinical Microbiology, 40*(11), 3951–3955.

Zhao, L., Gao, S., Huan, H., Xu, X., Zhu, X., Yang, W., et al. (2009). Comparison of virulence factors and expression of specific genes between uropathogenic Escherichia coli and avian pathogenic E. coli in a murine urinary tract infection model and a chicken challenge model. *Microbiology (Reading, England), 155*(Pt 5), 1634–1644. https://doi.org/10.1099/mic.0.024869-0

Chapter 13
Regulations in Poultry Meat Processing

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13.1 Introduction

Global production of poultry meat has been growing steadily since 2012, reaching record numbers in recent years (USDA 2017). Poultry is currently the second highest-consumed meat worldwide, and its global demand is projected to increase faster than other common meat types (USDA 2017). According to reports by the US Department of Agriculture (USDA 2017), the USA is the largest producer of broiler meat, accounting for more than 20% of all poultry production followed by Brazil, the European Union, and China. Furthermore, poultry meat has become an essential part of the diet among consumers in the USA, as the per capita consumption has doubled since 1980 (NCC 2017). This significant increase in consumption coupled with intensified production and processing practices has placed poultry as the most common food in disease outbreaks with confirmed pathogens in the USA (Chai et al. 2017).

The Centers for Disease Control and Prevention (CDC) governs the Foodborne Disease Outbreak Surveillance System (FDOSS), a surveillance system developed for the collection of data at a national, state, and local level regarding foodborne disease outbreaks. Recent reports from the CDC attribute most poultry-associated outbreaks with the consumption of raw or undercooked poultry contaminated with *Salmonella enterica*, *Campylobacter* spp., and *Clostridium perfringens* (Chai et al. 2017). In 2014, a chicken-associated multistate outbreak of multidrug-resistant *Salmonella* Heidelberg infections resulted in 634 illnesses and 241 hospitalizations (CDC 2014). Large outbreaks as the former increase the focus of poultry industry

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and the regulatory agencies on the improvement of safe poultry production and processing through continuous inspection and implementation of stringent pathogen reduction performance standards.

In the USA, the federal agency responsible for meat and poultry safety inspection and implementation of corresponding regulation is the US Department of Agriculture—Food Safety and Inspection Service (USDA-FSIS). In an ongoing effort to reduce the incidence of diseases associated with poultry consumption, the USDA-FSIS has established a set of regulations, guidelines, and performance standards aimed at reducing accidental contamination of poultry during slaughter and further processing. The current chapter encompasses a summary of poultry processing regulations from a food safety perspective to serve as guide for both processors and consumers.

13.2 Regulatory Acts and Enforcement in the Poultry Industry

13.2.1 Historical Background

Federal poultry inspection in the USA began in 1957, when the Congress passed the Wholesome Poultry Products Act, commonly known as the 1957 Poultry Products Inspection Act (PPIA). Novel scientific knowledge regarding communicable poultry diseases, coupled with an industry that was now raising over 1 billion broilers annually, was the key factor that resulted in the development of this act. The purpose of PPIA was to ensure that all poultry entering interstate or foreign commerce were not adulterated or misbranded, guaranteeing the commercialization of safe and wholesome poultry and poultry products. The Congress mandated through the 1957 Act that business slaughtering poultry or processing poultry products had to be federally inspected. In 1968 an amendment was passed, which called for cooperation between state and federal regulatory agencies. The 1968 Wholesome Poultry Products Act extended the existing mandate to all poultry slaughterhouses and processing plants that shipped their products within the state. This meant that all poultry slaughter and processing establishments in any state were subjected to federal regulations if state inspection requirements were not equivalent or more stringent than those stated in the PPIA.

The term “adulterated” was used in reference to poultry products that contained pesticides, chemicals, and other substances deemed detrimental to the consumers’ health. This also included poultry products carrying dirt and filth or prepared under unsanitary conditions. Federal inspectors would visually examine carcasses and internal organs for signs of disease, such as abnormal lymph nodes. If no visual evidence of any disease was found, the birds were considered appropriate for consumption. Additional inspection included the verification of refrigeration and

cooking temperatures, as well as supervision of plant sanitation and cleanup activities. Relying solely on organoleptic evaluation for identification of unsafe poultry products quickly proved to be an incompetent inspection system, as it was unable to detect or reduce the incidence of foodborne pathogens.

In 1996, the USDA-FSIS published the 1996 "Pathogen Reduction, Hazard Analysis and Critical Control Point (PR/HACCP) Systems" Final Rule. A new set of regulations for poultry processors designed and established to reduce the occurrence of foodborne pathogens in poultry products thereby limiting the incidence of foodborne illness among consumers of poultry meat was developed (USDA, 1996). Such requirements included the development and implementation of written standard operating procedures (SOPs) for sanitation, antimicrobial treatments, and carcass-cooling standards, consistent microbial testing for generic *Escherichia coli* as an indicator for fecal contamination, establishment of pathogen reduction performance standards for *Salmonella*, and, finally, the development and implementation of an HACCP plan. In 1998, the USDA-FSIS issued the "Poultry Post-mortem Inspection and Reinspection—Enforcing the Zero Tolerance for Visible Fecal Material" directive, in an effort to reduce the likelihood of cross-contamination during carcass processing (USDA, 1998). This zero-tolerance standard mandated that all carcasses must be free of visible fecal material, an indicator for contamination with *Enterobacteriaceae*, upon entering the chill tanks.

Implementation of the *Salmonella* Verification Program in 1996 prompted the development of new technologies for pathogen control in the food industry. The purpose of this program was to reduce the incidence of *Salmonella* in the final product by establishing a system that tests processing plants for *Salmonella* on a monthly basis, prioritizing pathogen testing in plants that fail to meet the established performance standards. This ensured the continuous evaluation of the implemented pathogen control and microbial testing programs. Changes with respect to the *Salmonella* Verification Program were introduced in 2011 (USDA, 2011), when the FSIS issued a Federal Register, the "New Performance Standards for *Salmonella* and *Campylobacter* in Young Chicken and Turkey Slaughter Establishment." The major changes included:

- Setting all poultry processing plants to the highest priority of testing schedule, triggered by the initiation of *Campylobacter* testing
- New *Salmonella* performance standards for broiler carcasses that would accept 9.8% positive samples or less and 7.1% positive for turkey carcasses
- New *Campylobacter* performance standards accepting 15.6% positive samples for broilers and 5.3% positive samples for turkeys

The FSIS has since then issued several regulations that set new pathogen reduction performance standards for the control of *Salmonella* and *Campylobacter* at the processing plant (USDA, 2011). In an effort to help poultry processors, the FSIS has also published guidelines for the development and performance of adequate microbial testing programs as well as recommendations for compliance with existing performance standards.

13.2.2 Current Regulation

The Office of Disease Prevention and Health Promotion (ODPHP) has issued a series of goals under the Healthy People 2020 initiative aimed at reducing the incidence of foodborne illnesses in the USA. The key objectives of this initiative are the reduction of foodborne outbreaks and infections caused by pathogens commonly transmitted through food, as well as increasing awareness among consumers and food processors on food safety practices that prevent such outbreaks. In an effort to meet these public health goals and effectively reduce the incidence of *Salmonella* and *Campylobacter* in poultry, the USDA-FSIS issued a final rule in 2014 titled "Modernization of Poultry Slaughter Inspection." This rule established a New Poultry Inspection System (NPIS) for broiler and turkey processing plants. The essential elements of this system included:

- The mandated removal of unsatisfactory carcasses and parts by qualified personnel before the arrival of federal inspector.
- Shifts in agency budgets that encourage offline inspection and verification activities, proven to be more effective in ensuring food safety than online inspection.
- Implementing and maintaining written procedures to prevent contamination of carcasses and parts by *Enterobacteriaceae* and fecal material during processing. By incorporating such procedures into their HACCP plans and monitoring them daily, the need for testing for generic *E. coli* was eliminated, allowing processing plants to use more relevant indicators for microbial control.
- Maintaining records which corroborate that their products meet the definition of ready-to-cook (RTC) poultry.
- Allowing processing plants to operate, when possible, at a maximum line speed of 140 bird per minute (bpm).

New performance standards for *Salmonella* and *Campylobacter* in raw minced chicken, chicken parts, and turkey products were proposed in 2015. These were finally implemented in 2017 with the issuance of the Federal Register "New Performance Standards for *Salmonella* and *Campylobacter* in Not-Ready-to-Eat Comminuted Chicken and Turkey Products and Raw Chicken Parts and Changes to Related Agency Verification Procedures." The new set of performance standards are shown in Table 13.1.

Table 13.1 *Salmonella* and *Campylobacter* performance standards (adapted from USDA 2016)

	Maximum acceptable percent positive		Performance standard	
Product	*Salmonella*	*Campylobacter*	*Salmonella*	*Campylobacter*
Comminuted chicken (325 g sample)	25.0	1.9	13 of 52	1 of 52
Comminuted turkey (325 g sample)	13.5	1.9	7 of 52	1 of 52
Chicken parts (4 lb sample)	15.4	7.7	8 of 52	4 of 52

The FSIS also mandated that establishments not meeting the proposed pathogen reduction performance standards, as well as establishments repetitively producing poultry products contaminated with antibiotic-resistant *Salmonella* or with *Salmonella* and *Campylobacter* strains matching those found in recent foodborne outbreaks or epidemiologically linked to disease, would have to be subjected to a scheduled Public Health Risk Evaluation (PHRE) and a Food Safety Assessment (FSA) based on results obtained by federal inspectors.

Regulatory requirements for the control of the deadly pathogen, *Listeria monocytogenes*, have also been developed for ready-to-eat (RTE) meat and poultry products. In 2003, the FSIS issued 9 CFR part 430, "Control of *Listeria monocytogenes* in Post-lethality Exposed Ready-to-Eat Products," better known as the *Listeria* Rule (USDA, 2003). A "zero tolerance" for the pathogen was implemented, and the rule mandated that all poultry establishments producing RTE poultry products include written procedures in their HACCP plan and SOPs to effectively control and reduce the overall incidence of the pathogen.

13.2.3 European Regulation

Animal production practices in the European Union differ significantly from those in the USA in relation to animal welfare, consumer health, and environmental contamination. Consumers in the European Union are highly concerned with how their food is produced and the impact that intensive food production systems have on the environment and public health. This integrative approach to food production has greatly influenced European legislation regarding food safety. A good example was Regulation 1831/2003/EC on additives for use in animal nutrition, an EU-wide ban on antibiotics as growth promoters in animal feed which entered into effect in 2006. This legislation was developed as a strategy to reduce the incidence of antimicrobial-resistant microbes in the food chain and prevent the dissemination of resistance to the public. While a trend shifting animal production to antibiotic-free practices exists in the USA, antibiotic use for growth promotion is still permitted.

A centralized system for the prevention and control of foodborne illnesses was implemented in the European Union with the development of Council Directive 92/117/EEC. The main purpose of the directive was to establish a monitoring system for the surveillance and protection against foodborne outbreaks, zoonoses, and antimicrobial resistance among zoonotic agents in the member states of the EU. For the poultry industry, Directive 92/117 encompassed a series of measures and control strategies against *Salmonella enteritidis* and *Salmonella typhimurium* infections in breeder flocks. The overall strategy to reduce the incidence of *Salmonella* in poultry and eggs was to produce pathogen-free chickens at breeder farms. Mandated slaughter of breeding flocks infected with *S. enteritidis* or *S. typhimurium* was one of the consequences of this directive.

In 2002, Regulation EC/178/2002, commonly known as General Food Law, was adopted by the European Parliament and the Council. The regulation established

"general principles, requirements, and procedures" regarding legislation of food and feed at a national and Union level. An integrative farm-to-fork approach to food safety was set, comprising all stages of the food chain, from production to retail sale. The general procedures consisted on:

- Applying the principles of risk analysis, through a science-based approach, to food production for the development of legislation. These principles are scientific risk assessment, risk management, and risk communication.
- Transparency at all stages in the development of food legislation, as well as protection of consumer interests through effective public communication about food safety.
- Traceability of all "foodstuffs, animal feed, food-producing animals, and all other materials meant to be consumed by humans or animals through all phases of production, processing and distribution" (EC/178/2002).

Regulation EC/178/2002 also established the formation of an independent agency, the European Food Safety Authority (EFSA), which assists the European Commission in matters of public health issues related to food consumption by providing scientific and technical information. EFSA, in collaboration with the European Centre for Disease Prevention and Control (ECDC), is also in charge of surveilling and monitoring foodborne outbreaks and prevalence of foodborne pathogens in foods. Through collection and analysis of data, EFSA is able to develop risk assessments and recommendations that will be used by member states for the implementation of pathogen reduction targets in foods or animals.

Currently, a general regulation for pathogen control in poultry is contained in Regulation (EC) No 2160/2003, which sets pathogen reduction targets and establishes measures for the control of *Salmonella* and other zoonotic agents (e.g., *Campylobacter*) at all stages of production, processing, and distribution. This heavily emphasizes the farm-to-fork approach by enhancing pathogen control at the level of primary production. Specific targets have been developed in recent years aimed to reduce the prevalence of *Salmonella*-infected laying hens of the *Gallus gallus* species, which can be found under Regulation (EC) No 200/2010. Equivalent regulation exists for laying hens (Regulation (EC) No 517/2011), broilers (Regulation (EC) No 200/2012), and turkeys (Regulation (EC) No 1190/2012), where their respective targets for pathogen reduction are specified.

13.3 Safety Management in the Processing Plant

Food safety assurance in the poultry processing industry is a multifactorial issue. Changes in environmental conditions (e.g., rises in ambient temperature and humidity), birds carrying high bacterial loads upon slaughter, and presence of fecal contamination during processing are key factors that can significantly reduce the effectiveness of pathogen control systems and ultimately compromise product safety. Microbiological hazards are present at all stages of the poultry meat production chain; a "farm-to-fork" approach is therefore necessary. Common poultry-associated

foodborne pathogens *Salmonella* and *Campylobacter* have reservoirs in healthy chickens from which they spread to humans, many times not causing illness in the infected host. Thus, the identification and control of such hazards can be challenging. The likelihood of carcass contamination during processing is quite high, as both *Salmonella* and *Campylobacter* can easily spread between birds and persist in many different areas of the processing plant. The goal for poultry processors and regulators is to reduce the incidence of disease pathogens in the final product to achievable minimums before the product reaches the consumer. This can only be achieved through implementation of a preventive, rather than reactive, food safety assurance system that evaluates food safety risks and contains hazards at each step of the processing chain.

13.3.1 The HACCP System and Prerequisite Programs

Development and implementation of the HACCP system in the food industry, as a preventive approach for food safety assurance, was first introduced in 1996, under the "Pathogen Reduction, Hazard Analysis and Critical Control Point (PR/HACCP) Systems" Final Rule issued by the USDA-FSIS. The HACCP system is regarded as the foundation in the industry's food safety assurance program. Through a science-based hazard analysis, the HACCP system surveys biological, chemical, and physical risks related to poultry processing, sets permissible limits, and develops management tools for processors to effectively contain the risks within the safety thresholds established. These thresholds, described as critical control points (CCPs), are designed to reduce or eliminate the identified hazards to acceptable levels at all stages of processing.

Implementation of a successful HACCP system relies heavily on well-designed prerequisite programs. Traditionally known as Good Manufacturing Practices (GMPs) and Standard Operating Procedures (SOPs), prerequisite programs are developed to manage food safety concerns of each segment of the food production establishment as well as process control. These programs are designed as written documents containing reliable and adequate procedures with specified operational parameters. When implemented, prerequisite programs should reduce the probability of food safety hazard development on an ongoing basis. Thus, these programs are essential for the successful implementation of a HACCP system, as they support the decision-making process during hazard analysis, ease process control, and facilitate management of the HACCP plan. As with hazard analysis and control, it is crucial that all prerequisite programs are subjected to recordkeeping in order to ensure that implemented practices are effective in hazard prevention. Prerequisite programs include, but are not limited to, cleaning and sanitation plans, pest control, recommended employee hygiene practices, and specifications of establishment facilities and equipment. Requirements on this subject can be found under the Code of Federal Regulations, 9 CFR Chapter III, Subchapter E—Regulatory Requirements under the Federal Meat Inspection Act and the Poultry Products Inspection Act.

13.3.2 Microbial Testing

Another essential tool for food safety management and compliance with pathogen reduction performance standards is the implementation of appropriate microbiological testing methods in accordance with a statistically valid sampling plan. An adequate sampling and analysis method aimed at detecting harmful microorganisms at defined locations and under established limits is therefore necessary to evaluate the effectiveness of an implemented HACCP plan. Under the New Poultry Inspection System (NPIS), established by the "Modernization of Poultry Slaughter Inspection" rule (USDA 2014), all poultry production establishments are required to include their microbiological sampling plans, as a written procedure, into their HACCP plan.

The 2015 FSIS Compliance Guide: Modernization of Poultry Slaughter Inspection—Microbiological Sampling of Raw Poultry (USDA 2015) was designed to help processors in the development and implementation of an appropriate sampling plan, as well as microbiological testing methodology. General recommendations on statistical process control, indicator organisms, sampling plan, analysis, and microbiological testing methods can be found in this guide.

13.4 Conclusions

Both the USA and EU have adopted a science-based preventive food safety approach for the production of safe and wholesome poultry products. The effectiveness of this approach relies greatly on collaborating efforts between monitoring agencies, regulators, and producers. Food safety is a public health issue of dynamic nature, which can only be targeted through continuous surveillance of potential and existing microbiological threats in the food production chain and reassessment of pathogen reduction performance standards. As a result, legislation should be constantly under review so that it can serve as a tool to collectively alleviate current and future hazards and mitigate the incidence of diseases at a global level.

References

Centers for Disease Control and Prevention. (2014). *Multistate outbreak of multidrug-resistant Salmonella Heidelberg infections linked to Foster Farms brand chicken (final update)*. Retrieved from https://www.cdc.gov/salmonella/heidelberg-10-13/

Chai, S. J., Cole, D., Nisler, A., & Mahon, B. E. (2017). Poultry: The most common food in outbreaks with known pathogens, United States, 1998–2012. *Epidemiology and Infection, 145*(2), 316–325.

Commission Regulation (EU) No 1190/2012 of 12 December 2012 concerning a Union target for the reduction of *Salmonella* Enteritidis and *Salmonella* Typhimurium in flocks of turkeys, as provided for in Regulation (EC) No 2160/2003 of the European Parliament and of the Council.

Commission Regulation (EU) No 200/2010 of 10 March 2010 implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards a Union target for the reduction of the prevalence of *Salmonella* serotypes in adult breeding flocks of *Gallus gallus*.

Commission Regulation (EU) No 200/2012 of 8 March 2012 concerning a Union target for the reduction of *Salmonella* Enteritidis and *Salmonella* Typhimurium in flocks of broilers, as provided for in Regulation (EC) No 2160/2003 of the European Parliament and of the Council.

Commission Regulation (EU) No 517/2011 of 25 May 2011 implementing Regulation (EC) No 2160/2003 of the European Parliament and of the Council as regards a Union target for the reduction of the prevalence of certain *Salmonella* serotypes in laying hens of *Gallus gallus* and amending Regulation (EC) No 2160/2003 and Commission Regulation (EU) No 200/2010.

Council Directive 92/117/EEC of 17 December 1992 concerning measures for protection against specified zoonosis and specified zoonotic agents in animals and products of animal origin in order to prevent outbreaks of food-borne infections and intoxications.

National Chicken Council (NCC). (2017). *Per capita consumption of poultry and livestock, 1965 to estimated 2018, in pounds*. Retrieved from http://www.nationalchickencouncil.org/about-the-industry/statistics/per-capita-consumption-of-poultry-and-livestock-1965-to-estimated-2012-in-pounds/

Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety.

Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition.

Regulation (EC) No 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of *Salmonella* and other specified food-borne zoonotic agents.

United States Department of Agriculture. (1996). Pathogen reduction: Hazard Analysis and Critical Control Point (HACCP) systems; final rule. Food Safety and Inspection Service. *Federal Register, 61*, 38806–38944.

United States Department of Agriculture. (1998). *Poultry post-mortem inspection and re-inspection-enforcing zero tolerance for visible fecal material. Directive 6150.1*. Washington, DC: Food Safety and Inspection Service.

United States Department of Agriculture. (2003). Control of *Listeria monocytogenes* in post-lethality exposed ready-to-eat products. Food Safety and Inspection Service. *Federal Register, 66*(34224), 652–653.

United States Department of Agriculture. (2011). New performance standards for *Salmonella* and *Campylobacter* in young chicken and turkey slaughter establishments: Response to comments and announcement of implementation schedule, notice. Food Safety and Inspections Service. *Federal Register, 76*, 15282–15290.

United States Department of Agriculture. (2014). Modernization of poultry slaughter inspection; final rule. Food Safety and Inspections Service. *Federal Register, 79*, 49566–49637.

United States Department of Agriculture. (2015). *FSIS Compliance Guideline: Modernization of poultry slaughter inspection- microbiological sampling of raw poultry*. Retrieved from https://www.fsis.usda.gov/wps/wcm/connect/a18d541e-77d2-40cf-a045-b2d2d13b070d/Microbiological-Testing-Raw-Poultry.pdf?MOD=AJPERES

United States Department of Agriculture. (2016). New performance standards for *Salmonella* and *Campylobacter* in not-ready-to-eat comminuted chicken and turkey products and raw chicken parts and changes to related agency verification procedures: Response to comments and announcement of implementation; notice. Food Safety and Inspections Service. *Federal Register, 81*, 7285–7300.

United States Department of Agriculture. (2017). *Livestock and poultry: World markets and trade*. Retrieved from https://apps.fas.usda.gov/psdonline/circulars/livestock_poultry.pdf