2017

Prevalence of avian pathogenic Escherichia coli and antibiotic resistance of E. coli isolates from the ceca of poultry fed Original XPC

Jasmine Carroll
Iowa State University

Prevalence of avian pathogenic *Escherichia coli* and antibiotic resistance of *E. coli* isolates from the ceca of poultry fed Original XPC™

by

Jasmine Carroll

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Microbiology

Program of Study Committee:
Mark Rasmussen, Major Professor
Steve Carlson
Nancy Cornick

Iowa State University

Ames, Iowa

2017

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

NOMENCLATURE

ExPEC	Extraintestinal Pathogenic *Escherichia coli*
UPEC	Uropathogenic *Escherichia coli*
NMEC	Neonatal Meningitis *Escherichia coli*
APEC	Avian Pathogenic *Escherichia coli*
UTI	Urinary Tract Infection
ESBL	Extended-Spectrum Beta-Lactamase
NDM	New Delhi metallo-Beta-Lactamase
MCR	Mobilized Colistin Resistance
PAI	Pathogenicity Island
SCFA	Short Chain Fatty Acid
FOS	Fructooligosaccharide
ROS	Reactive Oxygen Species
Epicor	Epicor®
XPC	Original XPC™
XLD	Xylose Lysine Deoxycholate
LB	Luria Broth

AKNOWLEDGEMENTS

I would like to extend thanks to my major professor, Dr. Mark Rasmussen, for continually guiding me through this journey, and my PI, Dr. Steve Carlson, for welcoming me into his lab and allowing me to pursue my interest in veterinary microbiology. Additionally, I would like to thank Dr. Nancy Cornick for taking the time as a member of my committee, supplying useful advice whenever needed.

A special thanks to Kristina Feye for guiding me through my thesis project and helping me through all the bumps in the road along the way. Also to Dr. Kristi Anderson for her assistance in the lab and her continuous words of encouragement. I will forever carry fond memories of our conversations and rants while processing samples.

Finally, I would like to thank the important people in my life who I could not have completed this journey without. My parents and sister are always there, providing emotional (and sometimes monetary) support whenever needed and always welcoming me back home with open arms. And a big shout out to my best friend Katie Lange. We started our educational journeys together and have supported each other through the good and the bad. I couldn't imagine the past seven years without my concert going, Disney loving, wine drinking buddy.

ABSTRACT

Avian pathogenic *Escherichia coli* (APEC) is an extra-intestinal pathogenic *E. coli* (ExPEC) pathotype that causes avian colibacillosis, resulting in a major economic loss to the poultry industry annually. It's not known to directly cause disease in humans, however APEC is able to transmit its plasmid-encoded virulence genes to human ExPEC pathotypes (UPEC and NMEC) or commensal microorganisms present in the human GI tract. This could potentially result in the creation of more virulent or antibiotic resistant strains and classifies APEC as potentially zoonotic. Increased regulation of antibiotic usage as growth promoters and disease preventatives in food animals has resulted in a push towards antibiotic alternatives. Original XPC^{TM} (Diamond V, Cedar Rapids) is a *Saccharomyces cerevisiae* fermentation product that has proved efficacious as a growth promoter and in reducing different pathogens across numerous animal species. Ceca from poultry provided a diet with this feed additive were collected at the time of slaughter, reconstituted in Luria Broth, plated on Xylose Lysine Deoxycholate selective and differential media, and *E. coli* colonies were assayed for the presence of APEC and subjected to an antibiogram. The presence of APEC in each cecum was determined utilizing a pentaplex PCR and individual *E. coli* isolates were tested for resistance against three antibiotics: ceftiofur, enrofloxacin, and chloramphenicol. Results revealed an overall reduction in both APEC prevalence and antibiotic resistance, supporting XPC as a useful alternative to antibiotics in the poultry industry.

CHAPTER 1: GENERAL INTRODUCTION

Thesis Introduction

Avian pathogenic *Escherichia coli* (APEC) is the leading cause of avian colibacillosis globally and is a major economic burden on the poultry industry due to the morbidity and mortality rates. APEC is classified as an extra-intestinal pathogenic *E. coli* (ExPEC) along with uropathogenic *E. coli* (UPEC) and neonatal meningitis *E. coli* (NMEC), the pathotypes known to cause disease in humans and mammals. Each ExPEC pathotype is known to cause a distinct disease depending on the location within the host, however they all have a common set of virulence genes located primarily on a colicin plasmid. Over recent years, these virulence genes have become associated with antibiotic resistance genes at an alarming frequency due to high recombination rates. Within the poultry industry this is a problem because it has increased the challenge of attempting to eliminate APEC from poultry flocks. APEC is also gaining further interest as a public health concern due to its ability to reside in the gastrointestinal tract of poultry as a commensal organism. The organism could potentially be consumed by the human population and act as a reservoir for virulence and resistance genes that can be transferred to other bacterial organisms within the human GI tract.

Given the importance of APEC in both the poultry industry and public health, it is imperative to find a method to control its prevalence in the poultry population to reduce disease and transference. The rapid increase in antibiotic resistance across most bacterial pathogens means this control method needs to be an alternative to antibiotics. This study focused on Original XPC^{TM} (Diamond V, Cedar Rapids), a *Saccharomyces cerevisiae*

fermentation product, that has proven efficacious in the reduction of gastrointestinal pathogens in numerous animal species.

This study aims to determine if XPC could be used as a prophylactic treatment for APEC in poultry. If successful it would reduce the morbidity and mortality rates seen in poultry, saving the industry millions of dollars. Additionally, it would mitigate the propagation of APEC and its virulence and antibiotic resistance genes. This could be important from a public health perspective to reduce the potential of creating more resistant human-associated *Enterobacteriaceae* pathogens capable of extra-intestinal infection. To accomplish the study goal, poultry fed a diet including XPC^{TM} were tested for the prevalence of APEC and antibiotic resistance of *E. coli* isolated from the cecum.

Thesis Organization

This thesis is organized into four chapters. Chapter 1 serves as the general introduction while Chapter 2 serves as the literature review covering topics relevant to the study of ExPEC. Topics covered in the literature review include a general introduction to ExPEC, colicin-encoding plasmids, antibiotic resistance, zoonotic potential of APEC, and alternatives to antibiotics. Chapter 3 is the original research chapter for the study testing the effects of Original XPC on APEC prevalence and antibiotic resistance of *E. coli* colonies isolated from poultry cecum. Chapter 4 includes general discussion, conclusions, and future directions.

The author's role in this study included assisting in development of the study design and culturing of *E. coli* from poultry cecum, collecting *E. coli* isolates for later assays, performing the laboratory assays, data collection and analysis, and manuscript writing.

CHAPTER 2: LITERATURE REVIEW ON EXTRAINTESTINAL PATHOGENIC *ESCHERICHIA COLI*

Escherichia coli (*E. coli*) is a member of the *Enterobacteriaceae* family and is commonly found in the environment, foods, and gastrointestinal tract of animals and humans (1). Commensal or pathogenic pathotypes are determined by the genome, which consists of a core genome and variable mobile genetic elements (2). The core genome is a conserved set of genes encoding the genetic information for essential cellular processes. Variable mobile genetic element is an umbrella term encompassing plasmids, transposons, prophages, and mobile genomic islands that encode strain-specific genetic information. Pathogenic *E. coli* exhibit a variety of pathotypes that are commonly characterized by somatic (O) and flagella (H) antigens (3). Different pathotypes are responsible for different disease syndromes, and are most commonly identified by their phenotypic disease characteristics. Despite their different disease presentation all *E. coli* pathotypes have the same basic scheme of pathogenesis; the organism must colonize the mucosal site, evade the host defense systems, replicate, and cause cytopathic effect. This review will focus on pathotypes characterized as extra-intestinal pathogenic *E. coli* (ExPEC), including classification, plasmid carriage, antibiotic resistance profile, zoonotic potential, as well as explore current alternatives to antibiotics.

Extra-intestinal Pathogenic *E. coli*

Classification of *E. coli* is important given the large number and variety of strains. Phylogenetic grouping is one method used with three assays available; multi-locus enzyme electrophoresis, ribotyping, or a triplex PCR assay (4–6). Four phylogenetic groups have been identified for *E. coli* (A, B1, B2, and D), within which certain pathotype patterns have

been observed (7). These phylogenetic groupings are indicative of a genetic divergence in the evolutionary history of *E. coli* that resulted in group B2 exhibiting more virulent tendencies due to the acquisition of virulence genes on plasmids, transposons, genetic islands, etc. (8). Serotyping is also commonly used in the classification of *E. coli* and is based on the 173 O (somatic), 80 K (capsular), and 56 H (flagellar) antigens (9). Determination of O and H antigens is by bacterial agglutination, while the K antigens are determined primarily by immunoprecipitation in a gel. The O antigens are based on the structure of the polysaccharide side chain present in the lipopolysaccharide in the surface of the *E. coli*, K antigens are related to the structures of acidic polysaccharides on the cell surface, and H antigens are related to the proteins that compose the flagella present on the *E. coli* cell. Numerous serotypes have been identified in the various pathotypes and strains of *E. coli*; some of these serotypes are unique to the pathotype while other serotypes are common across multiple pathotypes.

Many researchers have worked to identify traits to distinguish the three different pathotypes of ExPEC; uropathogenic *E. coli* (UPEC), neonatal meningitis *E. coli* (NMEC), and avian pathogenic *E. coli* (APEC) (10). One of the leading causes of urinary tract infections (UTIs) is UPEC, typically presenting as a superficial infection of the bladder and urinary tract, with about 4% of cases progressing to pyelonephritis (11, 12). Uncomplicated UTIs effect approximately 1 in 3 women by age 24 and cost the United States almost $2 billion annually (12, 13). NMEC is the second leading cause of neonatal meningitis globally, with a rate of 0.1 cases in every 1000 children born in the industrialized world (14, 15). Meningitis typically occurs between 6-9 days of age, has a mortality rate of 20-40%, and of those who survive approximately 50% develop neurological problems (15). Colibacillosis in

poultry is primarily a result of APEC infection, causing millions of dollars lost worldwide in the poultry industry due to significant morbidity and mortality rates (16, 17). In the United States, at least 30% of commercial flocks are affected by APEC with disease presentation ranging from salpingitis, peritonitis, swollen head syndrome, cellulitis, and necrotic dermatitis. While not a known cause of human disease, APEC is important to study as a potential human pathogen due to its numerous overlapping virulence characteristics with human ExPEC pathotypes.

Controversy remains as to whether these pathotypes are distinguishable from one another. Johnson et al. (16) identified 5 genes that, when all are present, are indicative of APEC, while Logue et al. (18) identified 10 potential discriminators of NMEC. However, there is an overlap of these genes within APEC, UPEC, and NMEC that questions the legitimacy of identifying them as individual pathotypes (19–22). Phylogenetic analysis emphasizes this understanding. The UPEC and NMEC strains most commonly fall into groups B2 and D, APEC strains fall into groups A and B2, while commensal strains fall primarily into group A (8, 19, 20, 23). Serotyping analyses indicate NMEC are most commonly classified within the O7 and O18 serogroups (20), UPEC within the O1, O2, O6, O15, O18, O25, O26, and O75 serogroups (19, 20, 23), and APEC within the O1, O2, O18, and O78 serogroups (24–26). Unfortunately, 10-40% of ExPEC strains are non-typeable, therefore serotyping is not a good indication of pathogenicity nor pathotype (19, 20, 23). The molecular analysis of the presence of mobile genetic elements signifies pathogenic potential more than broad phylogenic groups.

Animal colonization studies support the claim that these may not be unique pathotypes after all. In one study, chicken pulmonary sacs were experimentally infected with

UPEC strains and developed lesions similar to APEC. Further studies include experimentally inducing UTI in murine models with APEC, resulting in disease pathology associated with UPEC (23). A study from the same group also found that APEC isolates could cause sepsis and neonatal meningitis in a rat model, and that NMEC isolates were lethal to chick embryos and caused colisepticemia in chicks (27). These studies indicate that location may be more important for ExPEC than a designated pathotype. It is important to note that while all strains tested caused disease, mortality rates varied depending on the strain.

Infections caused by ExPEC strains are important to human and animal health, resulting in billions of dollars of lost due to health care costs and poultry mortality. Phylogenetic analysis and serotyping, although broad also have overlapping commonalities across ExPEC, revealing the close similarity of these pathotypes. This similarity is also exemplified in animal infection studies. As further analysis is done, ExPEC may need to be re-evaluated as one path-pathotype rather than three.

Colicin-Encoding Plasmids

An important feature of *E. coli* is the presence of numerous plasmids (extrachromosomal genetic elements) that confer different properties to the organism (28). A variety of plasmid sizes have been identified within *E. coli* with the tendency towards a bimodal distribution (fewer copies of large plasmids versus more copies of small plasmids) (29). The large, incompatible plasmids are typically the plasmids that encode virulence or resistance genes and are the largest contribution to variation in *E. coli* pathotypes (30). Transfer and recombination of these plasmids is a common occurrence, promoting genetic exchange between the genome and plasmids, ultimately leading to a complement of plasmids

and extensive genetic variation within individual bacterial chromosomes (31, 32). Despite the variation in genes carried on *E. coli* plasmids, it has been determined the majority of them have a common genetic backbone and therefore likely evolved from a single plasmid (30).

Invasive extra-intestinal *E. coli* are most commonly associated with the colicin-encoding plasmids (33, 34). Colicins are small molecules, produced by *Enterobacteriaceae,* that are classified as bacteriocins due to their antibacterial activity against related specie of bacteria (35). The ColV plasmid is the most common colicin-encoding plasmid and the primary contributor of virulence factors for the three ExPEC pathotypes (36–38). The original understanding of the plasmid purely encoding colicin production has evolved to include carriage of numerous virulence-encoding genes that are vital for extra-intestinal infection (34). Insertion sequences flanking the virulent genetic regions indicate that many of these traits likely originated in other pathogenic species and were included in the plasmid as a result of recombination (39–41). These molecular events resulted in the acquisition of traits such as iron sequestration (39, 42), iron and manganese transportation (43), increased serum survival (44), hemagglutination (40), and outer membrane vesicle production (45).

Plasmids are a defining feature of *Enterobacteriaceae* that provide variability in pathogenicity and resistance. Colicin-encoding plasmids harbor the virulence genes that define ExPEC strains and are therefore of the utmost importance to study and characterize. Identification of these virulence traits allows for elucidation of the detailed pathogenesis, which can be used to improve treatment and preventative measures of disease.

Molecular Identification of the APEC Pathotype

The APEC pathotype is conferred through ColV and ColBM plasmids, both encoding specific genes that aid in the virulence of the pathogen. The ColV plasmids are the primary plasmids identified as harboring ExPEC virulence traits, with numerous studies indicating the importance of this plasmid to the pathogen. Within the last decade, however a plasmid was identified in an APEC strain that also encodes these genes, the ColBM plasmid, which likely evolved from ColV (46). Importantly, the ColBM plasmid can confer antibiotic resistance genes as well as virulence genes associated with ColV (47). This exemplifies the prominence that recombination events within the colicin-encoding plasmids can have on the pathogenicity of these organisms. The conserved set of genes, commonly referred to as the pathogenicity island, located on the ColV plasmid is important for the successful transfer of virulence genes during recombination events (38). This pathogenicity island has been primarily located in APEC and the virulence genes within have been used as genetic identifiers for this pathotype (16). The importance of each virulence gene will now be discussed to understand their importance in the pathogenicity of ExPEC.

Iron is essential for the survival of bacteria due to its contribution to many cellular processes, including nucleotide biosynthesis, electron transport, and peroxide reduction (48). Mammalian hosts have limited amounts of free iron available as an innate mechanism of immunity to pathogenic bacteria through a complex system of proteins meant to sequester free iron (49). In response, pathogenic bacteria developed methods of iron sequestration in order to survive and replicate within the host (50). One common method of acquiring iron in deficient host environments is the production of siderophores, which are compounds released from the bacteria that competitively bind the iron that is bound by host molecules.

Siderophores can then bind to prokaryotic receptors and salvage host iron for use in molecular and biochemical processes essential for survival. The system is not perfect. For example, enteric bacteria produce siderophores referred to as enterochelins, that are inhibited by serum albumin, thus useless in systemic infections (51). To maximize iron salvaging capabilities, ExPEC encode different iron sequestration systems both in their genome and on plasmids found in APEC pathotypes. The two iron sequestration systems that have been located on the ColV plasmid are the aerobactin and salmochelin operons, which produce siderophores that are effective outside of the intestinal tract (30).

Siderophores produced by the aerobactin operon are hydroxamate compounds that have a transient association with the receptors on the bacterial membrane (42). The aerobactin operon region encodes five polypeptides. Four of the genes (*iucABCD*) encode polypeptides that participate in aerobactin synthesis and one gene (*iutA*) encodes an outer membrane protein that serves as a receptor (52, 53). Aerobactin has a lower affinity for iron compared to enterochelins, requiring it to preferentially scavenge iron from cells and tissues (54). The aerobactin operon is commonly found in most ExPEC, however there are variations in its encoded location. In animal strains, it is more frequently plasmid-encoded while in human strains it is most often located on the chromosome (34). Salmochelins are the second type of siderophore commonly found linked to the ColV plasmid and appear to contribute more to virulence than aerobactins (39, 55). The *iroA* locus is divided into two operons, *iroBCDE* and *iroN*, and encodes five genes responsible for synthesis, export, and import of salmochelins (56). Salmochelins are glycosylated variants of enterochelins, with the modification occuring within the bacteria by IroB and IroE enzymes. After modification, salmochelins are exported from the bacterial cell via the membrane-bound IroC and IroN

enzymes. Upon binding with an iron molecule, the siderophore-iron complex is imported by the same membrane enzymes and then degraded by IroD within the bacterial cell. Despite being located on plasmids, both the aerobactin and salmochelin encoding operons are regulated by the chromosomally located *fur* gene product, that represses production of the siderophores when efficient amounts of free iron are present in the environment (57, 58).

Ion transporters encoded on the ColV plasmid are also vital to ExPEC, particularly the *sitABCD* operon, encoding both iron and manganese transporters (43). The ABC-type transporters encoded by this operon have a higher affinity for iron, however the importance of manganese import cannot be diminished given its function as an antioxidant as well as a detoxifier of radical oxygen species, H_2O_2 and O_2^- (59). Researchers hypothesize that the use of manganese is important because it is a more energy efficient method of protecting the bacterial cell from radical oxygen species than superoxide dismutase and peroxidase enzymes. Regulation occurs via the same *fur* operon as the aerobactin and salmochelin operons, which is primarily repressed by iron but also by manganese in some cases (60).

Commonly coupled with the *sit* operon is *iss*, which encodes a polypeptide that increases survival of the pathogenic *E. coli* in host serum (41, 61). Host serum has numerous bactericidal components, the most notable being lysozymes and complement that act on the bacterial surface to cause cell lysis (62). For ExPEC to cause systemic infection, it is therefore important to produce a protein able to counteract these serum components. Three variations of *iss* have been identified; type 1 is primarily located on ColV plasmids and most common in APEC and NMEC isolates, while type 3 has primarily been discovered on the chromosome of UPEC isolates (41). Currently nothing is known about the mechanism of the gene product, although due to its high homology with a similar chromosomally encoded

polypeptide involved in serum survival, it is believed to be a membrane-bound molecule (61).

The final two important virulence genes to briefly identify and discuss are *hlyF* and *tsh*. HlyF was originally believed to be a product associated with hemolytic activity (63). This characterization was deemed to be inaccurate when studies revealed its involvement in the formation of outer membrane vesicles (OMVs) (45). The OMVs release toxins and induce autophagy of eukaryotic cells, an important contribution to the cytopathic effect of ExPEC. The *tsh* associated with ColV plasmids, encodes a temperature sensitive hemagglutinin that has hemagglutination activity on chicken erythrocytes predominately at about 26ºC (40, 64, 65). Studies done in APEC revealed Tsh may have additional virulence contributions, such as acting as an adhesin, increasing the rate of colonization in the airsacs, and contributing to lesions and fibrin deposition in the air sacs of poultry (40). Studies evaluating Tsh have primarily been done on APEC, therefore not much is currently known about the contribution of Tsh in the virulence of other ExPEC pathotypes.

The conserved pathogenicity island of the ColV plasmid houses several virulence genes crucial to extra-intestinal infections, encoding traits ranging from iron sequestration and transportation to serum survival and outer membrane vesicle formation. The genes located on this pathogenicity island have proved useful as predictors of APEC; however, the easy transferability of these virulence genes is of great concern.

Zoonotic Potential of APEC

Zoonotic organisms, by definition, are organisms that normally reside in animal species but can transmit to and infect humans (66). Controversy exists around the zoonotic

potential of APEC pathotypes. Two arguments are put forward to suggest that APEC should be considered a potential concern to human health are that: 1) they are reservoirs for promiscuous antibiotic resistance plasmids (67) and 2) the pathotype causes experimental disease in multiple hosts (23, 27). Therefore, the ColV and ColBM plasmids are insidious contributors to the zoonotic potential of APEC (30). The APEC associated plasmid pAPEC-O2-ColV has been transferred to an avian commensal *E. coli* and successfully conferred pathogenicity within chicken and mice models (68). Commensal *E. coli* became lethal to chicken embryos upon acquisition of the plasmid and in other studies exhibited enhanced growth in human urine, and colonization in the kidney of a murine model (68). These results exemplify the potential of APEC as a zoonotic pathogen for humans, combined with the knowledge that APEC can colonize the GI tract of poultry (69).

Additional evidence for APEC and the colicin plasmids as a public health concern is seen in the identification of a plasmid in a *Salmonella* serovar and drug resistant *E. coli* isolates in avian organic fertilizer (70–72). *Salmonella enterica* serovar Kentucky is a pathogen that has been increasingly identified as a causative agent of disease in poultry, humans, and companion animals; further, it has recently been found to carry the conserved pathogenicity island of the ColV plasmid (70, 71). The carriage of the ColV-like plasmid in *S*. Kentucky has been determined to increase colonization capabilities and virulence of the pathogen in extra-intestinal regions in poultry (70). This plasmid contributes to antibiotic resistance through the carriage of tetracycline resistance (71). Low levels of tetracycline in a host or the environment has been shown to increase the occurrence of conjugative transfer of *tetR* encoded plasmids (73). Given the regular use of antibiotics in animal production (74), the dissemination and transfer of the ColV plasmid and its virulence genes is likely being

propagated by the industry. These plasmids are subject to vertical transmission through the food chain through the utilization of organic fertilizers from avian compost. Fertilizers produced from organic animal waste are commonly used for vegetable production and undergo a composition process thought to reduce the presence of potential microbial pathogens found in the avian excrement (75). Sixty percent of *E. coli* isolates from fertilizer were identified as having at least one gene from the ColV plasmid conserved pathogenicity island (72). Of all the isolates tested, 50% of them were resistant to at least one antibiotic, with the most common ones being tetracycline, amoxicillin, ampicillin, and streptomycin. This indicates that the composting process is not completely successful at removing *E. coli* encoding APEC virulence and antibiotic resistance genes. These strains could therefore be acquired by humans via consumption and handling of produce.

The zoonotic potential of APEC is exemplified by the appearance of pColV virulence genes and resistance genes in alternative genera and the environment. Presence of APEC within the GI tract of poultry sent to slaughter is of concern as a source of not only a potentially human pathogen, but also a reservoir of virulence and antibiotic resistance genes capable of high rates of transmission and recombination. From a public health stand point, a prophylactic treatment needs to be identified to mitigate the transmission of APEC to humans.

Antibiotic Resistance

Antibiotic resistance is a problem in pathogenic microorganisms that was first identified in the 1930s and is a significant threat to clinical and veterinary health worldwide (76). While development of antibiotic resistance in microorganisms is a natural occurrence (77), humans have accelerated the process over the last fifty years due to non-therapeutic an

inappropriate therapeutic use of antibiotics (78). Thus, resulting in upwards of millions of metric tons of antibiotics released into the environment (76). There are many methods in which microorganisms can acquire antibiotic resistance genes, but the most concerning is plasmid-mediated transmission. When bacteria are exposed to low levels of antibiotics in their environment, an SOS response is activated that enhances plasmid transmission and recombination (76, 79). The continuous use and release of antibiotics in the environment is therefore perpetuating the spread of antibiotic resistance genes.

Enterobacteriaceae as a family are a reservoir of plasmids bearing antibiotic resistance genes undergoing frequent conjugation and transformation (80–82). Accordingly, the high usage of antibiotics in production animals as prophylaxis and growth promoters has led to an increase in antibiotic resistance in APEC found in poultry. Results from data demonstrate significantly increased resistance to sulfamethoxazole, tetracycline, streptomycin, ampicillin, ciprofloxacin, penicillin, tylosin, and enrofloxacin (24, 83–86). Resistance of APEC to many of these drugs has been linked to the acquisition of resistance-encoding genes, primarily through plasmid recombination (87). As these mobile genetic elements are vertically transmitted through the food chain (88–90), the prodigious use of antibiotics in veterinary medicine has significantly contributed to the problem.

The human clinical impact of this characteristic is far reaching and deeply concerning world-wide. Not only is there significant acquisition of mobile genetic elements from the environment and through vertical transmission in the food chain, the misuse of antibiotics in clinical settings significantly amplifies this threat. As of 2010, up to 45% of ExPEC isolates from clinical patients were determined to be resistant to cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole, all of which are commonly prescribed for Gram

negative infections (91). This increase in resistance within human ExPEC pathotypes is likely a result of overprescribing antibiotics for the treatment of uncomplicated urinary tract infections (92) and for intrapartum use in females (93).

Genotypes conferring resistance pathotypes are diverse. Many isolates of ExPEC have recently been classified as carriers of extended-spectrum β lactamases (ESBLs) which provide the pathogen with resistance to a wide variety of β lactams, including 3rd and 4th generation cephalosporins and penicillins (94, 95). Irrespective of host, it has been determined that CTX-M is the most prevalent ESBL worldwide (96), and has been identified on both narrow and broad host-range plasmids (97). Strains of *E. coli* encoded for CTX-M hydrolyze penicillins, cephalosporins, and monobactams (98). Fluoroquinolone resistance is also commonly identified in CTX-M encoding isolates due to the presence of closely linked plasmid-mediated *qnr* genes (97). Additional genes that have been located on a plasmid harboring CTX-M encode resistance to aminoglycosides, chloramphenicol, sulfonamide, trimethoprim, and tetracycline (99); classifying many CTX-M carrying isolates as multi-drug resistant.

The New Delhi metallo-β-lactamase (NDM-1) is another important β-lactamase that has appeared in *E. coli* within the last decade, enabling resistance to all β-lactams (91, 100). NDM-1 has been identified in a UPEC isolate in association with a plasmid belonging to sequence type 131 (ST131) (101). This is an important relationship to understand as more than 50% of ST131 clones have been associated with multi-drug resistance in ExPEC and almost 70% associated with fluoroquinolone and extended-spectrum cephalosporin resistance (102). Analysis of ST131-positive *E. coli* has also revealed that they tend to combine both

resistance and virulence for increased pathogenesis that was not commonly seen in isolates in the past.

Finally, *mcr-1*, a colistin resistance-encoding gene, has spread globally within a few years (103–106). Colistin is of the Polymyxin E antibiotic class and is of great concern to public health because of its classification as a last resort antibiotic in the treatment of multi-drug resistant infections (103, 107). Since its appearance a few years ago, *mcr-1* has already been identified in ExPEC isolates worldwide (103, 104, 108–110). The first *E. coli* harboring *mcr-1* in the United States was isolated from a patient with a UTI in 2016 (109). This colistin resistance gene was located on a novel IncF plasmid that was identified as also encoding CTX-M and tetracycline resistance. The location of these resistance genes on a plasmid type well-known for dissemination of genes among *Enterobacteriaceae* (111) is of great concern for public health worldwide.

Antibiotic resistance has seen dramatic increases in recent decades, largely due to the occurrence of resistance genes located on transmissible plasmids. Inappropriate use of antibiotics in feed animals, even at low levels, has escalated the rate of the natural transmission process, resulting in the large number of multi-drug resistant pathogens seen today. ExPEC has been associated with plasmids housing genes encoding several ESBLs, including CTX-M, NDM-1, and MCR-1. Identification of resistance to colistin, a last resort drug, within APEC has further exemplified the need to reduce the prevalence of this gene reservoir within poultry.

Alternatives to Antibiotics

With the rise of antibiotic resistance in microbial pathogens, there has been a surge in research to identify drugs or natural compounds that can prevent and reduce disease development in both animals and humans. Recent identification of the involvement of the gut microbiota in the regulation of host metabolic pathways (112) has led much of the research to focus on developing a prophylactic treatment that supports a healthy gut microbiome. Many of these treatment options try to optimize growth and development of beneficial bacteria and compounds that are already present in a healthy GI tract. Three categories of prophylactic treatments that have been developed are prebiotics, probiotics, and postbiotics.

Prebiotics are classified as non-digestible food ingredients that improve the health of the host through the promotion of growth or activity of beneficial bacteria present in the colon (113). Prebiotics are typically defined by the presence of β-linkages in the cell wall that enable the prebiotic to survive the enzymatic degradation process of the upper GI tract (114). In general, any indigestible fiber can be classified as a prebiotic, but only two prebiotics thus far meet all the criteria. Inulins and fructooligosaccharides (FOS) are fermented by certain species and strains of lactic acid bacteria and *Bifidobacterium*, promoting the growth of these bacterial species and increasing the production of short chain fatty acids (SCFAs) (113, 114). Specifically, fermentation of inulin results in the production of butyrate and fermentation of FOS produces acetate and lactic acid. Colonocytes in the cecum and large intestine utilize the SCFAs for energy, with butyrate being the most important in this instance (115). Additionally, SCFAs contribute to maintaining luminal pH acidity, modulating hormone secretion, activating fatty acid oxidation, regulating lipid and

glucose metabolism, and participating in anti-inflammatory processes (115, 116). It has also been determined that butyrate is capable of downregulating genes in the *Salmonella* pathogenicity island (117), reducing the potential virulence of *Salmonella* species. This is one indication of the importance of butyrate in the GI tract.

Probiotics are live microorganisms added to feed or ingested in a capsule with the intention of promoting the health of the GI tract and the host as a whole (118). The primary genus used in probiotics currently is *Lactobacillus* due to their association with a healthy GI tract (119). *Lactobacillus* spp. contribute to a healthy GI tract via reduction of intestinal permeability and the reduction of pathogenic bacteria due to lactic acid, bacteriocins, and hydrogen peroxide production and competitive exclusion. Probiotics are available for both human and animal use; however, inconsistent results indicate probiotics may need to be more specialized for animal species and their overall efficacy is debated (119). In fact, a recent study determined that probiotics can have negative effects, inducing local inflammation in healthy hosts and in hosts with inflammatory bowel disease detrimentally effecting the inflamed tissue (120). Results from this same study revealed that in a tissue model postbiotics are more effective at reducing inflammation caused by *Salmonella* than probiotics.

Postbiotics are soluble metabolites produced by probiotic microorganisms that have been shown to have beneficial effects on the health of the GI tract, including bacteriocin effects, improvement of the integrity of the mucosal gut barrier, and modulation of inflammatory mediator secretion (121). The most common postbiotics studied thus far have been metabolites derived from *Lactobacillus* species, with similar results seen across numerous animal species including rats, broiler chickens, laying hens, and swine (122–125).

Metabolites are most commonly included in the feed of the animal, and studies have determined they are most effective when a variety of metabolites isolated from different strains of *Lactobacillus plantarum* are included (123, 124). Common benefits from the ingestion of these metabolites include a decrease in fecal pH, decrease in prevalence of *Enterobacteriaceae* in the feces contrasted with an increase in lactic acid bacteria, increase in the concentration of SCFAs present in the feces, and an increase in the height of the intestinal villi (122–125). In piglets, these factors contributed to an overall increase in growth performance, indicated by birth weight, overall weight gain, average weight gain per day, and reduction in diarrhea production (124). A similar study in laying hens found that postbiotics contributed to a reduction in plasma and yolk cholesterol within eggs (122). This is an important benefit to humans that consume chicken eggs because cholesterol is known to be a leading cause of coronary heart disease (126). Overall the numerous health benefits that have been identified from the use of postbiotics classifies them as favorable alternatives to antibiotics, with further research being done to identify additional benefits as well as other organisms that can provide beneficial metabolites.

Recent research has focused on *Saccharomyces cerevisiae* fermentation products as a promoter for health in humans and animals. Currently there are two yeast fermentation-based products at the top of the market; EpiCor® (EpiCor) produced by Embria Health Services as a supplement for humans and Original XPC™ (XPC) produced by Diamond V as a feed additive for animals. These postbiotics are a result of anaerobic fermentation of *S. cerevisiae* in a proprietary medium, followed by drying of the liquid in order to obtain the yeast metabolites (127). Numerous studies have tested the efficacy of these products on the gut microbiota, host immune response, and growth response in production animals. In vitro

studies indicate that XPC-adapted chicken ceca are better able to inhibit *Salmonella* colonization and result in increased concentrations of acetate and butyrate present (128). EpiCor studies have shown similar results, in addition to antioxidant and anti-inflammatory activity in human leukocytes (127, 129).

In vivo studies have been vital in testing the efficacy of XPC, especially in production animals, the setting in which it has the potential to make a large impact in the replacement of antibiotics as growth additives. Broiler chickens fed XPC in their diet exhibit a significant reduction in shedding and large intestinal colonization of *Salmonella* Typhimurium, as well as a reduction in tissue culture invasiveness and chloramphenicol resistance of the *Salmonella* colonies isolated from those chickens (130). Weaned pigs have an overall improvement of growth performance, potentially due to the increased jejunal villi width and area allowing for enhanced digestion and absorption of nutrients in the intestine (131). When the sows are fed XPC during gestation and lactation they produce litters with greater weight and require fewer recovery days between weaning and the next breeding cycle (132). In *Salmonella* challenged pre-weaned dairy calves provided XPC in milk replacer and feed, there was a reduction in diarrhea and fever as well as improved development of the rumen (133). The current research clearly indicates the benefits *Saccharomyces cerevisiae* fermentation products have on animal health, with further research still being done to test the effects on other gastrointestinal pathogens and antibiotic resistance across multiple species.

Conclusion

Bacterial diseases such as urinary tract infections, neonatal meningitis, and avian colibacillosis are most commonly caused by ExPEC pathotypes. These pathogens are well defined by the presence of a ColV or ColBM plasmid that harbors a large majority of the

core virulence genes. The high transfer and recombination rate of these virulence and resistance plasmids has led ExPEC pathotypes to become a global public health concern. The potential of APEC as a zoonotic pathogen is exemplified by the new virulent *Salmonella* strain due to acquisition of ColV plasmid-encoded genes (70, 71) and a ColBM plasmid encoding macrolide resistance (47). Additionally, APEC strains can cause both urinary tract infections and meningitis in rat models when inoculated in the appropriate location for disease to occur (23, 27). It is therefore possible that APEC strains colonizing the poultry GI tract (134) could be consumed by humans and potentially cause human infections. Even if APEC consumed from poultry does not result in disease, cells that survive the passage to the intestine will have the opportunity to transmit and recombine plasmids, potentially leading to the development of pathogenic organisms that were once commensal or more virulent and/or drug resistant APEC strains.

Given the potential APEC has as a zoonotic pathogen to humans and the large economic impact it has on the poultry industry, it is important to find a method of reducing its prevalence within poultry. The increase in antibiotic resistance is leading to a reduction in the use of antibiotics as a disease preventative and growth promoter in feed animals. Original XPC™ is a postbiotic that is currently widely used and already has many proven benefits across multiple animal species (128, 130–132). The fermented *Saccharomyces cerevisiae* product is produced by Diamond V and is provided to animals as a feed additive. Proven benefits within poultry include increased body weight, increased daily weight gain, reduction in both *Salmonella* Typhimurium and *Campylobacter coli*, and a decrease in *Salmonella* virulence and antibiotic resistance (130, 135). Research should be done to

evaluate the efficacy on APEC prevalence and antibiotic resistance, to determine if XPC is a good alternative to antibiotics for the poultry industry.

References

1. **Dirk Van Elsas J**, **Semenov A V**, **Costa R**, **Trevors JT**. 2011. Survival of *Escherichia coli* in the environment: fundamental and public health aspects. ISME J **580**:173–183.
2. **Dobrindt U**. 2005. (Patho-)Genomics of *Escherichia coli*. Int J Med Microbiol **295**:357–371.
3. **Kaper JB**, **Nataro JP**, **Mobley HLT**. 2005. Pathogenic *Escherichia coli*. Nat Rev **2**:123–140.
4. **Selander RK**, **Musser JM**, **Caugant DA**, **Gilmour MN**, **Whittam TS**. 1987. Population genetics of pathogenic bacteria. Microb Pathog **3**:1–7.
5. **Bingen EH**, **Denamur E**, **Elion J**. 1994. Use of ribotyping in epidemiological surveillance of nosocomial outbreaks. Clin Microbiol Rev **7**:311–327.
6. **Clermont O**, **Bonacorsi S**, **Bingen E**, **Bonacorsi P**. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol **66**:4555–4558.
7. **Herzer PJ**, **Inouye S**, **Inouye M**, **Whittam TS**. 1990. Phylogenetic distribution of branched RNA-linked multicopy single-stranded-DNA among natural isolates of *Escherichia coli*. J Bacteriol **172**:6175–6181.
8. **Picard B**, **Garcia JS**, **Gouriou S**, **Duriez P**, **Brahimi N**, **Bingen E**, **Elion J**, **Denamur E**. 1999. The link between phylogeny and virulence in *Escherichia coli* extraintestinal infection. Infect Immun **67**:546–553.
9. **Orskov F**, **Orskov I**. 1992. *Escherichia coli* serotyping and disease in man and animals. Can J Microbiol **38**:699–704.
10. **Russo TA**, **Johnson JR**. 2000. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. J Infect Dis **181**:1753–1754.
11. **Foxman B**, **Brown P**. 2003. Epidemiology of urinary tract infections. Infect Dis Clin North Am **17**:227–241.
12. **Rosenberg M**. 1999. Pharmacoeconomics of treating uncomplicated urinary tract infections. Int J Antimicrob Agents **11**:247–251.
13. **Foxman B**, **Barlow R**, **D'Arcy H**, **Gillespie B**, **Sobel JD**. 2000. Urinary tract infection: Self-reported incidence and associated costs. Ann Epidemiol **10**:509–515.

14. **May M**, **Daley AJ**, **Donath S**, **Isaacs D**. 2005. Early onset neonatal meningitis in Australia and New Zealand, 1992-2002. Arch Dis Child Fetal Neonatal Ed **90**:F324-327.

15. **Bonacorsi S**, **Bingen E**. 2005. Molecular epidemiology of *Escherichia coli* causing neonatal meningitis. Int J Med Microbiol **295**:373–381.

16. **Johnson TJ**, **Wannemuehler Y**, **Doetkott C**, **Johnson SJ**, **Rosenberger SC**, **Nolan LK**. 2008. Identification of minimal predictors of avian pathogenic *Escherichia coli* virulence for use as a rapid diagnostic tool. J Clin Microbiol **46**:3987–3996.

17. **Dho-moulin M**, **Fairbrother JM**. 1999. Avian pathogenic *Escherichia coli* (APEC). Vet Res **30**:299–316.

18. **Logue CM**, **Doetkott C**, **Mangiamele P**, **Wannemuehler YM**, **Johnson TJ**, **Tivendale KA**, **Li G**, **Sherwood JS**, **Nolan LK**. 2012. Genotypic and phenotypic traits that distinguish neonatal meningitis-associated *Escherichia coli* from fecal *E. coli* isolates of healthy human hosts. Appl Environ Microbiol **78**:5824–5830.

19. **Rodriguez-Siek KE**, **Giddings CW**, **Doetkott C**, **Johnson TJ**, **Fakhr MK**, **Nolan LK**. 2005. Comparison of *Escherichia coli* isolates implicated in human urinary tract infection and avian colibacillosis. Microbiology **151**:2097–2110.

20. **Ewers C**, **Li G**, **Wilking H**, **Kießling S**, **Alt K**, **Antáo EM**, **Laturnus C**, **Diehl I**, **Glodde S**, **Homeier T**, **Böhnke U**, **Steinrück H**, **Philipp HC**, **Wieler LH**. 2007. Avian pathogenic, uropathogenic, and newborn meningitis-causing *Escherichia coli*: How closely related are they? Int J Med Microbiol **297**:163–176.

21. **Johnson TJ**, **Kariyawasam S**, **Wannemuehler Y**, **Mangiamele P**, **Johnson SJ**, **Doetkott C**, **Skyberg JA**, **Lynne AM**, **Johnson JR**, **Nolan LK**. 2007. The genome sequence of avian pathogenic *Escherichia coli* strain O1:K1:H7 shares strong similarities with human extraintestinal pathogenic *E. coli* genomes. J Bacteriol **189**:3228–3236.

22. **Peigne C**, **Bidet P**, **Mahjoub-Messai F**, **Plainvert C**, **Barbe V**, **Médigue C**, **Frapy E**, **Nassif X**, **Denamur E**, **Bingen E**, **Bonacorsi S**. 2009. The plasmid of *Escherichia coli* strain S88 (O45:K1:H7) that causes neonatal meningitis is closely related to avian pathogenic *E. coli* plasmids and is associated with high-level bacteremia in a neonatal rat meningitis model. Infect Immun **77**:2272–2284.

23. **Zhao L**, **Gao S**, **Huan H**, **Xu X**, **Zhu X**, **Yang W**, **Gao Q**, **Liu X**. 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 **155**:1634–1644.

24. **Zhao S**, **Maurer JJ**, **Hubert S**, **De Villena JF**, **McDermott PF**, **Meng J**, **Ayers S**, **English L**, **White DG**. 2005. Antimicrobial susceptibility and molecular characterization of avian pathogenic *Escherichia coli* isolates. Vet Microbiol **107**:215–224.

25. **Ewers C**, **Janßen T**, **Kießling S**, **Philipp HC**, **Wieler LH**. 2004. Molecular epidemiology of avian pathogenic *Escherichia coli* (APEC) isolated from colisepticemia in poultry. Vet Microbiol **104**:91–101.

26. **Rodriguez-Siek KE**, **Giddings CW**, **Doetkott C**, **Johnson TJ**, **Nolan LK**. 2005. Characterizing the APEC pathotype. Vet Res **36**:241–256.

27. **Tivendale KA**, **Logue CM**, **Kariyawasam S**, **Jordan D**, **Hussein A**, **Li G**, **Wannemuehler Y**, **Nolan LK**. 2010. Avian-pathogenic *Escherichia coli* strains are similar to neonatal meningitis *E. coli* strains and are able to cause meningitis in the rat model of human disease. Infect Immun **78**:3412–3419.

28. **Actis La**, **Tolmasky ME**, **Crosa JH**. 1999. Bacterial plasmids: Replication of extrachromosomal genetic elements encoding resistance to antimicrobial compounds. Front Biosci **4**:D43–D62.

29. **Sherratt DJ**. 1974. Plasmids Review. Cell **3**:189–195.

30. **Johnson TJ**, **Nolan LK**. 2009. Pathogenomics of the virulence plasmids of *Escherichia coli*. Microbiol Mol Biol Rev **73**:750–74.

31. **Smillie C**, **Garcillan-Barcia MP**, **Francia M V**, **Rocha EP**, **de la Cruz F**. 2010. Mobility of plasmids. Microbiol Mol Biol Rev **74**:434–452.

32. **West SC**. 1992. Enzymes and molecular mechanisms of genetic recombination. Annu Rev Biochem **61**:603–640.

33. **Smith HW**. 1974. A search for transmissible pathogenic characters in invasive strains of *Escherichia coli*: the discovery of a plasmid-controlled toxin and a plasmid-controlled lethal character closely associated, or identical, with colicin V. J Gen Microbiol **83**:95–111.

34. **Waters VL**, **Crosa JH**. 1991. Colicin V virulence plasmids. Microbiol Rev **55**:437–450.

35. **Cascales E**, **Buchanan SK**, **Duche D**, **Kleanthous C**, **Lloubes R**, **Postle K**, **Riley M**, **Slatin S**, **Cavard D**. 2007. Colicin biology. Microbiol Mol Biol Rev **71**:158–229.

36. **Nicholson BA**, **West AC**, **Mangiamele P**, **Barbieri N**, **Wannemuehler Y**, **Nolan LK**, **Logue CM**, **Li G**. 2016. Genetic characterization of ExPEC-like virulence plasmids among a subset of NMEC. PLoS One **11**:1–16.

37. **DebRoy C**, **Sidhu MS**, **Sarker U**, **Jayarao BM**, **Stell AL**, **Bell NP**, **Johnson TJ**. 2010. Complete sequence of pEC14_114, a highly conserved IncFIB/FIIA plasmid associated with uropathogenic *Escherichia coli* cystitis strains. Plasmid **63**:53–60.

38. **Johnson TJ**, **Siek KE**, **Johnson SJ**, **Nolan LK**. 2006. DNA Sequence of a ColV plasmid and prevalence of selected plasmid-encoded virulence genes among avian *Escherichia coli* strains. J Bacteriol **188**:745–758.

39. **Sorsa LJ**, **Dufke S**, **Heesemann J**, **Schubert S**. 2003. Characterization of an iroBCDEN gene cluster on a transmissable plasmid of uropathogenic *Escherichia coli*: Evidence for horizontal transfer of a chromosomal virulence factor. Infect Immun **71**:3285–3293.

40. **Dozois CM**, **Dho-Moulin M**, **Brée A**, **Fairbrother JM**, **Desautels C**, **Curtiss R**. 2000. Relationship between the Tsh autotransporter and pathogenicity of avian *Escherichia coli* and localization and analysis of the tsh genetic region. Infect Immun **68**:4145–4154.

41. **Johnson TJ**, **Wannemuehler YM**, **Nolan LK**. 2008. Evolution of the iss gene in *Escherichia coli*. Appl Environ Microbiol **74**:2360–2369.

42. **Warner PJ**, **Williams PH**, **Bindereif A**, **Neilands JB**. 1981. ColV plasmid-specific aerobactin synthesis by invasive strains of *Escherichia coli*. Infect Immun **33**:540–5.

43. **Sabri M**, **Léveillé S**, **Dozois CM**. 2006. A SitABCD homologue from an avian pathogenic *Escherichia coli* strain mediates transport of iron and manganese and resistance to hydrogen peroxide. Microbiology **152**:745–758.

44. **Binns MM**, **Mayden J**, **Levine RP**. 1982. Further characterization of complement resistance conferred on *Escherichia coli* by the plasmid genes traT of R100 and iss of ColV, I-K94. Infect Immun **35**:654–659.

45. **Murase K**, **Martin P**, **Porcheron G**, **Houle S**, **Helloin E**, **Penary M**, **Nougayrede JP**, **Dozois CM**, **Hayashi T**, **Oswald E**. 2016. HlyF produced by extraintestinal pathogenic *Escherichia coli* is a virulence factor that regulates outer membrane vesicle biogenesis. J Infect Dis **212**:856–865.

46. **Johnson TJ**, **Johnson SJ**, **Nolan LK**. 2006. Complete DNA sequence of a ColBM plasmid from avian pathogenic *Escherichia coli* suggests that it evolved from closely related ColV virulence plasmids. J Bacteriol **188**:5975–5983.

47. **Johnson TJ**, **Jordan D**, **Kariyawasam S**, **Stell AL**, **Bell NP**, **Wannemuehler YM**, **Fernández-Alarcón C**, **Li G**, **Tivendale KA**, **Logue CM**, **Nolan LK**. 2010. Sequence analysis and characterization of a transferable hybrid plasmid encoding multidrug resistance and enabling zoonotic potential for extraintestinal *Escherichia coli*. Infect Immun **78**:1931–1942.

48. **Neilands JB**. 1991. A brief history of iron metabolism. Biol Met **4**:1–6.

49. **Skaar EP**. 2010. The battle for iron between bacterial pathogens and their vertebrate hosts. PLoS Pathog **6**:1–2.

50. **Ratledge C**, **Dover LG**. 2000. Iron metabolism in pathogenic bacteria. Annu Rev Microbiol **54**:881–941.

51. **Konopka K**, **Neilands JB**. 1984. Effect of serum albumin on siderophore-mediated utilization of transferrin iron. Biochemistry **23**:2122–2127.

52. **Carbonetti NH**, **Williams PH**. 1984. A cluster of five genes specifying the aerobactin iron uptake system of plasmid ColV-K30. Infect Immun **46**:7–12.

53. **Gross R**, **Engelbrecht F**, **Braun V**. 1984. Genetic and biochemical characterization of the aerobactin synthesis operon on pColV. MGG Mol Gen Genet **196**:74–80.

54. **Brock JH**, **Williams PH**, **Liceaga J**, **Wooldridge KG**. 1991. Relative availability of transferrin-bound iron and cell-derived iron to aerobactin-producing and enterochelin-producing strains of *Escherichia coli* and to other microorganisms. Infect Immun **59**:3185–3190.

55. **Gao Q**, **Wang X**, **Xu H**, **Xu Y**, **Ling J**, **Zhang D**, **Gao S**, **Liu X**. 2012. Roles of iron acquisition systems in virulence of extraintestinal pathogenic *Escherichia coli*: salmochelin and aerobactin contribute more to virulence than heme in a chicken infection model. BMC Microbiol **12**:143-154.

56. **Fischbach M a**, **Lin H**, **Liu DR**, **Walsh CT**. 2006. How pathogenic bacteria evade mammalian sabotage in the battle for iron. Nat Chem Biol **2**:132–138.

57. **Braun V**, **Burkhardt R**. 1982. Regulation of the ColV plasmid-determined iron(III) aerobactin transport system in *Escherichia coli*. J Bacteriol **152**:223–231.

58. **Balbontín R**, **Villagra N**, **Pardos de la Gándara M**, **Mora G**, **Figueroa-Bossi N**, **Bossi L**. 2016. Expression of IroN, the salmochelin siderophore receptor, requires mRNA activation by RyhB small RNA homologues. Mol Microbiol **100**:139–155.

59. **Horsburgh MJ**, **Wharton SJ**, **Karavolos M**, **Foster SJ**. 2002. Manganese: Elemental defence for a life with oxygen? Trends Microbiol **10**:496–501.

60. **Ikeda JS**, **Janakiraman A**, **Kehres DG**, **Maguire ME**, **Slauch JM**. 2005. Transcriptional regulation of sitABCD of *Salmonella enterica* serovar Typhimurium by MntR and Fur. J Bacteriol **187**:912–922.

61. **Horne SM**, **Pfaff-McDonough SJ**, **Giddings CW**, **Nolan LK**. 2000. Cloning and sequencing of the iss gene from a virulent avian *Escherichia coli*. Avian Dis **44**:179–184.

62. **Moll A**, **Manning PA**, **Timmis KN**. 1980. Plasmid-determined resistance to serum bactericidal activity: A major outer membrane protein, the traT gene product, is responsible for plasmid-specified serum resistance in *Escherichia coli*. Infect Immun **28**:359–367.

63. **Morales C**, **Lee MD**, **Hofacre C**, **Maurer JJ**. 2004. Detection of a novel virulence gene and a *Salmonella* virulence homologue among *Escherichia coli* isolated from broiler chickens. Foodborne Pathog Dis **1**:160–165.

64. **Provence DL**, **Curtiss R 3rd**. 1994. Isolation and characterization of a gene involved in hemagglutination by an avian pathogenic *Escherichia coli* strain. Infect Immun **62**:1369–1380.

65. **Stathopoulos C**, **Provence DL**, **Curtiss R**. 1999. Characterization of the avian pathogenic *Escherichia coli* hemagglutinin Tsh, a member of the immunoglobulin a protease-type family of autotransporters. Infect Immun **67**:772–781.

66. **Bidaisee S**, **Macpherson CNL**. 2014. Zoonoses and one health : A review of the literature. J Parasit Research **2014**:1-8.

67. **Couce A**, **Blázquez J**. 2009. Side effects of antibiotics on genetic variability. FEMS Microbiol Rev **33**:531–538.

68. **Skyberg JA**, **Johnson TJ**, **Johnson JR**, **Clabots C**, **Logue CM**, **Nolan LK**. 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. Infect Immun **74**:6287–6292.

69. **Ewers C**, **Antão EM**, **Diehl I**, **Philipp HC**, **Wieler LH**. 2009. Intestine and environment of the chicken as reservoirs for extraintestinal pathogenic *Escherichia coli* strains with zoonotic potential. Appl Environ Microbiol **75**:184–192.

70. **Johnson TJ**, **Thorsness JL**, **Anderson CP**, **Lynne AM**, **Foley SL**, **Han J**, **Fricke WF**, **Mcdermott PF**, **White DG**, **Khatri M**, **Stell AL**, **Flores C**, **Singer RS**. 2010. Horizontal gene transfer of a colV plasmid has resulted in a dominant avian clonal type of *Salmonella* enterica serovar kentucky. PLoS One **5**:1–10.

71. **Fricke WF**, **McDermott PF**, **Mammel MK**, **Zhao S**, **Johnson TJ**, **Rasko DA**, **Fedorka-Cray PJ**, **Pedroso A**, **Whichard JM**, **LeClerc JE**, **White DG**, **Cebula TA**, **Ravel J**. 2009. Antimicrobial resistance-conferring plasmids with similarity to virulence plasmids from avian pathogenic *Escherichia coli* strains in *Salmonella* enterica serovar Kentucky isolates from poultry. Appl Environ Microbiol **75**:5963–5971.

72. **Gazal LES**, **Puño-Sarmiento JJ**, **Medeiros LP**, **Cyoia PS**, **da Silveira WD**, **Kobayashi RKT**, **Nakazato G**. 2015. Presence of pathogenicity islands and virulence genes of extraintestinal pathogenic *Escherichia coli* (ExPEC) in isolates from avian organic fertilizer. Poult Sci **94**:3025–3033.

73. **Salyers AA**, **Shoemaker NB**. 1996. Resistance gene transfer in anaerobes: New insights, new problems **23**:36–43.

74. **FDA**. 2015. Antimicrobials sold or distributed for use in food-producing Animals. FDA Rep.

75. **Bernal MP**, **Alburquerque JA**, **Moral R**. 2009. Composting of animal manures and chemical criteria for compost maturity assessment. A review. Bioresour Technol **100**:5444–5453.

76. **Davies J**, **Davies D**. 2010. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev **74**:417–433.

77. **Gottlieb D**. 1976. The production and role of antibiotics in soil. J Antibiot (Tokyo) **29**:987–1000.

78. **Doyle MP**, **Busta F**, **Cords BR**, **Davidson PM**, **Al. E**. 2006. Antimicrobial resistance: Implications for the food system. Compr Rev Food Sci Food Saf **5**:71–137.

79. **Gullberg E**, **Cao S**, **Berg OG**, **Ilbäck C**, **Sandegren L**, **Hughes D**, **Andersson DI**. 2011. Selection of resistant bacteria at very low antibiotic concentrations. PLoS Pathog **7**:1–9.

80. **Casewell MW**, **Phillips I**. 1981. Aspects of the plasmid-mediated antibiotic resistance and epidemiology of *Klebsiella* species. Am J Med **70**:459–462.

81. **Galimand M**, **Guiyoule A**, **Gerbaud G**, **Rasoamanana B**, **Chanteau S**, **Carniel E**, **Courvalin P**. 1997. Multidrug resistance in *Yersinia pestis* mediated by a transferrable plasmid. Massachusetts Med Soc **337**:677–680.

82. **Boyd EF**, **Hartl DL**. 1997. Recent horizontal transmission of plasmids between natural populations of *Escherichia coli* and *Salmonella enterica*. J Bacteriol **179**:1622–1627.

83. **Yang H**, **Chen S**, **White DG**, **Zhao S**, **McDermott P**, **Walker R**, **Meng J**. 2004. Characterization of multiple-antimicrobial-resistant *Escherichia coli* isolates from diseased chickens and swine in China. J Clin Microbiol **42**:3483–3489.

84. **Diarrassouba F**, **Diarra MS**, **Bach S**, **Delaquis P**, **Pritchard J**, **Topp E**, **Skura BJ**. 2007. Antibiotic resistance and virulence genes in commensal *Escherichia coli* and *Salmonella* isolates from commercial broiler chicken farms. J Food Prot **70**:1316–1327.

85. **Obeng AS**, **Rickard H**, **Ndi O**, **Sexton M**, **Barton M**. 2012. Antibiotic resistance, phylogenetic grouping and virulence potential of *Escherichia coli* isolated from the faeces of intensively farmed and free range poultry. Vet Microbiol **154**:305–315.

86. **Radwan IA-E**, **Sayed H**, **Salam H**, **Abd-alwanis SAA**. 2014. Frequency of some virulence associated genes among multidrug-resistant *Escherichia coli* isolated from septicemic broiler chicken. Int J Adv Res **2**:867–874.

87. **Pitout JD**. 2012. Extraintestinal pathogenic *Escherichia coli*: an update on antimicrobial resistance, laboratory diagnosis and treatment. Expert Rev Anti Infect Ther **10**:1165–1176.

88. **Manges AR**, **Smith SP**, **Lau BJ**, **Nuval CJ**, **Eisenberg JNS**, **Dietrich PS**, **Riley LW**. 2007. Retail meat consumption and the acquisition of antimicrobial resistant *Escherichia coli* causing urinary tract infections: A case–control study. Foodborne Pathog Dis **4**:419–431.

89. **Overdevest I**, **Willemsen I**, **Rijnsburger M**, **Eustace A**, **Xu L**, **Hawkey P**, **Heck M**, **Savelkoul P**, **Vandenbroucke-Grauls C**, **van der Zwaluw K**, **Huijsdens X**, **Kluytmans J**. 2011. Extended-spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. Emerg Infect Dis **17**:1216–1222.

90. **Dutil L**, **Irwin R**, **Finley R**, **Ng LK**, **Avery B**, **Boerlin P**, **Bourgault AM**, **Cole L**, **Daignault D**, **Desruisseau A**, **Demczuk W**, **Hoang L**, **Horsman GB**, **Ismail J**, **Jamieson F**, **Maki A**, **Pacagnella A**, **Pillai DR**. 2010. Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. Emerg Infect Dis **16**:48–54.

91. **Pitout JDD**. 2012. Extraintestinal pathogenic *Escherichia coli*: A combination of virulence with antibiotic resistance. Front Microbiol **3**:1–7.

92. **Gupta K**, **Hooton TM**, **Naber KG**, **Wullt B**, **Colgan R**, **Miller LG**, **Moran GJ**, **Nicolle LE**, **Raz R**, **Schaeffer AJ**, **Soper DE**. 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. Clin Infect Dis **52**:103–120.

93. **Bizzarro MJ**, **Dembry L-M**, **Baltimore RS**, **Gallagher PG**. 2008. Changing patterns in neonatal *Escherichia coli* sepsis and ampicillin resistance in the era of intrapartum antibiotic prophylaxis. Pediatrics **121**:689–696.

94. **Pitout JD**, **Laupland KB**. 2008. Extended-spectrum beta-lactamase-producing *Enterobacteriaceae*: An emerging public-health concern. Lancet Infect Dis **8**:159–166.

95. **Shaikh S**, **Fatima J**, **Shakil S**, **Rizvi SMD**, **Kamal MA**. 2015. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi J Biol Sci **22**:90–101.

96. **Giufrè 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. J Antimicrob Chemother **67**:860–867.

97. **Cantón R**, **Coque TM**. 2006. The CTX-M beta-lactamase pandemic. Curr Opin Microbiol **9**:466–475.

98. **Paterson DL**, **Bonomo RA**. 2005. Extended-spectrum beta-lactamases : A clinical update. Clin Microbiol Rev **18**:657–686.

99. **Bonnet R**. 2004. Growing group of extended spectrum: the CTX-M enzymes. Antimicrob Agent Chemother **48**:1–14.

100. **Rogers B a**, **Sidjabat HE**, **Silvey A**, **Anderson TL**, **Perera S**, **Li J**, **Paterson DL**. 2013. Treatment options for New Delhi metallo-beta-lactamase-harboring *Enterobacteriaceae*. Microb Drug Resist **19**:100–103.

101. **Peirano G**, **Schreckenberger PC**, **Pitout JDD**. 2011. Characteristics of NDM-1-producing *Escherichia coli* isolates that belong to the successful and virulent clone ST131. Antimicrob Agents Chemother **55**:2986–2988.

102. **Johnson JR**, **Johnston B**, **Clabots C**, **Kuskowski MA**, **Castanheira M**. 2010. *Escherichia coli* sequence type ST131 as the major cause of serious multidrug-resistant *E. coli* infections in the United States. Clin Infect Dis **51**:286–294.

103. **Liu YY**, **Wang Y**, **Walsh TR**, **Yi LX**, **Zhang R**, **Spencer J**, **Doi Y**, **Tian G**, **Dong B**, **Huang X**, **Yu LF**, **Gu D**, **Ren H**, **Chen X**, **Lv L**, **He D**, **Zhou H**, **Liang Z**, **Liu JH**, **Shen J**. 2016. Emergence of plasmid-mediated colistin resistance mechanism mcr-1 in animals and human beings in China: A microbiological and molecular biological study. Lancet Infect Dis **16**:161–168.

104. **Hasman H**, **Hammerum AM**, **Hansen F**, **Hendriksen RS**, **Olesen B**, **Agersø Y**, **Zankari E**, **Leekitcharoenphon P**, **Stegger M**, **Kaas RS**, **Cavaco LM**, **Hansen DS**, **Aarestrup FM**, **Skov RL**. 2015. Detection of mcr-1 encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, denmark 2015. Eurosurveillance **20**:1–5.

105. **Falgenhauer L**, **Waezsada SE**, **Yao Y**, **Imirzalioglu C**, **Käsbohrer A**, **Roesler U**, **Michael GB**, **Schwarz S**, **Werner G**, **Kreienbrock L**, **Chakraborty T**. 2016. Colistin resistance gene mcr-1 in extended-spectrum β-lactamase-producing and carbapenemase-producing Gram-negative bacteria in Germany. Lancet Infect Dis **16**:282–283.

106. **Meinersmann RJ**, **Ladely SR**, **Plumblee JR**, **Cook KL**, **Thacker E**. 2017. Prevalence of mcr-1 in the cecal contents of food animals in the United States **61**:1–4.

107. **Michalopoulos AS**, **Tsiodras S**, **Rellos K**, **Mentzelopoulos S**, **Falagas ME**. 2005. Colistin treatment in patients with ICU-acquired infections caused by multiresistant Gram-negative bacteria: The renaissance of an old antibiotic. Clin Microbiol Infect **11**:115–121.

108. **Perreten V**, **Strauss C**, **Collaud A**, **Gerber D**. 2016. Colistin resistance gene mcr-1 in avian-pathogenic *Escherichia coli* in South Africa. Antimicrob Agents Chemother **60**:4414–4415.

109. **McGann P**, **Snesrud E**, **Maybank R**, **Corey B**, **Ong AC**, **Clifford R**, **Hinkle M**, **Whitman T**, **Lesho E**, **Schaecher KE**. 2016. *Escherichia coli* harboring *mcr-1* and *bla* $_{\text{CTX-M}}$ on a novel IncF plasmid: First report of *mcr-1* in the USA. Antimicrob Agents Chemother **60**:4420-4421.

110. **Barbieri NL**, **Nielsen DW**, **Wannemuehler Y**, **Cavender T**, **Hussein A**, **Yan S**, **Nolan LK**, **Logue CM**. 2017. mcr-1 identified in avian pathogenic *Escherichia coli* (APEC). PLoS One **12**:e0172997.

111. **Villa L**, **García-Fernández A**, **Fortini D**, **Carattoli A**. 2010. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. J Antimicrob Chemother **65**:2518–2529.

112. **Nicholson JK**, **Holmes E**, **Kinross J**, **Burcelin R**, **Gibson G**, **Jia W**, **Pettersson S**. 2012. Host-gut microbiota metabolic interactions. The Gut Microbiota **108**:1262–1268.

113. **Roberfroid M**. 2007. Prebiotics: The concept revisited. J Nutr **137**:830S–837S.

114. **Ricke SC**. 2015. Potential of fructooligosaccharide prebiotics in alternative and nonconventional poultry production systems. Poult Sci **94**:1411–1418.

115. **den Besten G**, **van Eunen K**, **Groen AK**, **Venema K**, **Reijngoud DJ**, **Bakker BM**. 2013. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res **54**:2325–2340.

116. **Vinolo MAR**, **Rodrigues HG**, **Nachbar RT**, **Curi R**. 2011. Regulation of inflammation by short chain fatty acids. Nutrients **3**:858–876.

117. **Gantois I**, **Ducatelle R**, **Pasmans F**, **Haesebrouck F**, **Thompson A**, **Hinton JC**, **Immerseel F Van**, **Hautefort I**. 2006. Butyrate specifically down-regulates *Salmonella* pathogenicity island 1 gene expression. Appl Environ Microbiol **72(1)**:946–949.

118. **Fuller R**. 1989. Probiotics in man and animals. J Appl Bacteriol **66**:365–378.

119. **Yeoman CJ**, **White BA**. 2014. Gastrointestinal tract microbiota and probiotics in production animals. Annu Rev Anim Biosci **2**:469–486.

120. **Tsilingiri K**, **Barbosa T**, **Penna G**, **Caprioli F**, **Sonzogni A**, **Viale G**, **Rescigno M**. 2012. Probiotic and postbiotic activity in health and disease: Comparison on a novel polarised ex-vivo organ culture model. Gut **61**:1007–1015.

121. **Cicenia A**, **Scirocco A**, **Carabotti M**, **Pallotta L**, **Marignani M**, **Severi C**. 2014. Postbiotic activities of Lactobacilli-derived factors. J Clin Gastroenterol **48**:18–22.

122. **Loh TC**, **Choe DW**, **Foo HL**, **Sazili AQ**, **Bejo MH**. 2014. Effects of feeding different postbiotic metabolite combinations produced by Lactobacillus plantarum strains on egg quality and production performance, faecal parameters and plasma cholesterol in laying hens. BMC Vet Res **10**:149.

123. **Loh TC**, **Thanh NT**, **Foo HL**, **Hair-Bejo M**, **Azhar BK**. 2010. Feeding of different levels of metabolite combinations produced by *Lactobacillus plantarum* on growth performance, fecal microflora, volatile fatty acids and villi height in broilers. Anim Sci J **81**:205–214.

124. **Thu T V.**, **Loh TC**, **Foo HL**, **Yaakub H**, **Bejo MH**. 2011. Effects of liquid metabolite combinations produced by *Lactobacillus plantarum* on growth performance, faeces characteristics, intestinal morphology and diarrhoea incidence in postweaning piglets. Trop Anim Health Prod **43**:69–75.

125. **Loh TC**, **Chong SW**, **Foo HL**, **Law FL**. 2009. Effects on growth performance, faecal microflora and plasma cholesterol after supplementation of spray-dried metabolite to postweaning rats. Czech J Anim Sci **54**:10–16.

126. **Weggemans RM**, **Zock PL**, **Katan MB**. 2001. Dietary cholesterol from eggs increases the ratio of total cholesterol to high-density lipoprotein cholesterol in humans: A meta-analysis. Am J Clin Nutr **73**:885–891.

127. **Jensen GS**, **Hart AN**, **Schauss AG**. 2007. An antiinflammatory immunogen from yeast culture induces activation and alters chemokine receptor expression on human natural killer cells and B lymphocytes in vitro. Nutr Res **27**:327–335.

128. **Rubinelli P**, **Roto S**, **Kim SA**, **Park SH**, **Pavlidis HO**, **McIntyre D**, **Ricke SC**. 2016. Reduction of *Salmonella* Typhimurium by fermentation metabolites of Diamond V Original XPC in an in vitro anaerobic mixed chicken cecal culture. Front Vet Sci **3(83)**:1-7.

129. **Possemiers S**, **Pinheiro I**, **Verhelst A**, **Van Den Abbeele P**, **Maignien L**, **Laukens D**, **Reeves SG**, **Robinson LE**, **Raas T**, **Schneider YJ**, **Van De Wiele T**, **Marzorati M**. 2013. A dried yeast fermentate selectively modulates both the luminal and mucosal gut microbiota and protects against inflammation, as studied in an integrated in vitro approach. J Agric Food Chem **61**:9380–9392.

130. **Feye KM**, **Anderson KL**, **Scott MF**, **McIntyre DR**, **Carlson SA**. 2016. Inhibition of the virulence, antibiotic resistance, and fecal shedding of multiple antibiotic-resistant *Salmonella* Typhimurium in broilers fed Original XPC^{TM}. Poult Sci 2902–2910.

131. **Price KL**, **Totty HR**, **Lee HB**, **Utt MD**, **Fitzner GE**, **Yoon I**, **Ponder MA**, **Escobar J**. 2010. Use of *Saccharomyces cerevisiae* fermentation product on growth performance and microbiota of weaned pigs during *Salmonella* infection. J Anim Sci **88**:3896–3908.

132. **Kim SW**, **Brandherm M**, **Freeland M**, **Newton B**, **Cook D**, **Yoon I**. 2008. Effects of yeast culture supplementation to gestation and lactation diets on growth of nursing piglets. Asian-Australasian J Anim Sci **21**:1011–1014.

133. **Brewer MT**, **Anderson KL**, **Yoon I**, **Scott MF**, **Carlson SA**. 2014. Amelioration of salmonellosis in pre-weaned dairy calves fed *Saccharomyces cerevisiae* fermentation products in feed and milk replacer. Vet Microbiol **172**:248–255.

134. **Dziva F**, **Stevens MP**. 2008. Colibacillosis in poultry: unravelling the molecular basis of virulence of avian pathogenic *Escherichia coli* in their natural hosts. Avian Pathol **37**:355–66.

135. **Hofacre CL**, **Mathis GF**, **Mcintyre DR**, **Broomhead JP**. 2015. Effects of Original XPC on cecal colonization by *Campylobacter coli* in broiler chickens : Preliminary Report. PO **225**:1–8.

CHAPTER 3: PREVALENCE OF AVIAN PATHOGENIC *ESCHERICHIA COLI* AND ANTIBIOTIC RESISTANCE OF *E. COLI* ISOLATES FROM THE CECA OF POULTRY FED ORIGINAL XPC™

Abstract

Avian pathogenic *Escherichia coli* (APEC), is an extra-intestinal pathogenic *E. coli* (ExPEC) pathotype. It is the leading cause of avian colibacillosis and results in significant economic loss to the poultry industry annually. While APEC is not a proven cause of disease in humans, it does transmit its plasmid-encoded virulence and resistance genes to *Enterobacterieaceae*. Horizontal gene transfer is well known for resulting in increased antibiotic resistance and pathogenic conversion of commensal microorganisms. Increased regulation of antibiotics as growth promoters and disease preventatives creates a void for antibiotic alternatives. Original XPC™ (Diamond V, Cedar Rapids) is a *Saccharomyces cerevisiae* fermentation product originally used as a growth promoter. Evidence has emerged indicating significant potential for the reduction of the prevalence of multiple pathogens. To evaluate the effect of XPC on APEC, ceca from broiler poultry on XPC containing diets (treatment) or standard industry corn-soy based meal (control) were evaluated for changes in antibiotic resistance and the APEC pathotype. Treatment with XPC resulted in a reduction in the prevalence of APEC by 50% in 10/13 of the poultry sources tested. Antibiotic resistance in XPC-treated poultry exhibited an almost 2-fold reduction for ceftiofur, enrofloxacin, and chloramphenicol. These results, combined with the results of previous studies, support XPC as a useful alternative antibiotic in the poultry industry.

Introduction

Avian pathogenic *Escherichia coli* (APEC) is a pathotype of extra-intestinal pathogenic *E. coli* (ExPEC), which are the cause of neonatal meningitis and urinary tract infections in humans (1). The avian pathotype is the causative agent of colibacillosis and septicemia in birds that leads to localized inflammation, most commonly presenting as perihepatitis, airsacculitis, and/or pericarditis. It can also be fatal. Importantly, APEC colonizes the gastrointestinal tract of poultry, and accordingly is an opportunistic pathogen capable of causing disease in immunosuppressive states (2). APEC is easily aerosolized from feces and thus threatens poultry housed together (1). Further, APEC is inherently resistant to many common disinfectants, making management difficult (3). This results in significant morbidity and mortality rates up to 20% and millions of dollars lost in the industry worldwide (4, 5), requiring a pressing need to find a management solution.

In addition to the impact on the poultry industry, APEC also has potential as a zoonotic pathogen due its shared set of virulence genes with human ExPEC pathotypes, uropathogenic *E. coli* (UPEC) and neonatal meningitis *E. coli* (NMEC) (6–8). The pathotype UPEC is one of the leading causative agents of urinary tract infections (9) and NMEC the second leading cause of neonatal meningitis worldwide (10). Interestingly, APEC strains can cause urinary tract infections and meningitis in rat models, and UPEC and NMEC strains can cause colibacillosis in poultry (11, 12). These studies suggest that APEC inadvertently transferred from poultry has the potential to cause disease in humans. Further, due to the nature of *Enterobacteriaceae*, APEC acts as a reservoir of virulence and antimicrobial resistance genes that are easily transferrable to other *E. coli* strains or Gram-negative species residing in the microbiota. Previous studies have revealed that acquisition of a pColV by

commensal *E. coli* results in the ability of the organism to cause lethality in chicken embryos, enhanced in vitro growth in human urine, and colonization of the kidney in a murine model (13).

A colicin plasmid, pColV, contains a majority of the core virulence genes that are characteristic of ExPEC pathogenesis (14). With the common occurrence of recombination among plasmids and bacterial chromosomes (15), these virulence genes have the potential to be transferred to not only other *E. coli* but also other bacterial species. This is exemplified in the identification of ColV plasmid-linked virulence genes in a strain of *Salmonella enterica* serovar Kentucky that increase colonization capabilities of the pathogen and increase virulence in extra-intestinal regions of the poultry (16).

A concerning feature of APEC resides in the antimicrobial resistance genes associated with non-colicin and colicin plasmids (17). Plasmid mediated antibiotic resistance within ExPEC isolates thus far include ESBL carriage, including CTX-M (18, 19), NDM-1 (20, 21), as well as MCR-1 (22). *Enterobacteriaceae* serves as a potent reservoir for these plasmids and enhances the concern of the spread of antimicrobial resistance. This rise of antibiotic resistance in not only ExPEC but many other human and animal pathogens has forced an evaluation of the use of antibiotics, especially within food animal production (23). Antibiotics are commonly used for growth promotion and disease prophylaxis. The void left requires immediate investigation into potent alternatives to antibiotics that act as both efficacious prophylaxis and growth promoters.

Yeast fermentation products from *Saccharomyces cerevisiae* have recently been at the forefront of this research endeavor due to largely efficacious results across multiple food animals. The benefits include reduction of pathogenic bacteria, modification of intestinal

morphology to improve nutrient absorption, enhancement of the immune system, and reduction of energy required for intestinal epithelial maintenance (24–27). The fermentation of the yeast produces beneficial metabolites while reducing the potentially negative effects of live cultures. These metabolites produced include carotenoids, vitamins, enzymes, amino acids, and some yet uncharacterized products that appear to be beneficial to host health. Original XPC™ (Diamond V, Cedar Rapids), has emerged as a relevant postbiotic. The evaluation of the efficacy of XPC in poultry revealed an increase in finisher weight, decrease in feed to gain ratios, a reduction in *Salmonella* Typhimurium and *Campylobacter coli* colonization and shedding, and a decrease in *Salmonella* virulence and antibiotic resistance (24, 25, 28). Given that *Salmonella enterica* and *E. coli* are genetically at least 95% similar (29), it is reasonable to hypothesize that XPC might have similar effects on APEC within poultry.

Currently the best method available for identifying APEC is the pentaplex PCR assay designed by Johnson et al (4). For *E. coli* to be classified as APEC, all 5 ColV plasmid-associated virulence genes must be present: *iroN*, *ompT*, *hlyF*, *iss*, and *iutA*. Respectively, these genes encode a salmochelin siderophore receptor (*iroN*) (30), a putative outer membrane protein (*ompT*) (31), a protein involved in outer membrane vesicle formation (*hlyF*) (32), a polypeptide that increases serum survival (*iss*) (33), and an aerobactin siderophore receptor (*iutA*) (34).

It is currently unknown as to if XPC decreases APEC virulence and colonization in poultry. To evaluate changes in APEC carriage, the pentaplex PCR assay was performed on *E. coli* cultured from the ceca of broiler chickens and turkeys from thirteen poultry factories across the United States. Additionally, antibiotic resistance was evaluated using an

antibiogram on individual *E. coli* isolates from poultry ceca. Results indicate an overall reduction in the prevalence of APEC in poultry fed an XPC-based diet compared to those on a standard diet.

Materials and Methods

Study Design

Ceca were collected on site from poultry headed to slaughter from thirteen different poultry sources across the United States; 5 sources provided turkey ceca, 7 sources provided broiler chicken ceca, and 1 source provided cloacal swabs from layer chickens. At each source, birds were fed either an XPC containing diet (treatment) or standard industry corn-soy based meal (control). Over a 15-month period ceca were shipped overnight and received in Whirl-Pak® bags on ice in groups of 25-100, each group representing a poultry barn fed either the XPC-based diet or the control diet. At the time of the study all lab employees remained uninformed to the classification of the barns.

E. coli Isolation

To evaluate the colonization of poultry with APEC, turkey or chicken ceca were weighed, ligated, and contents were resuspended in Luria Broth (LB). The contents were massaged to release the cecal contents and aliquoted onto Xylose Lysine Deoxycholate (XLD) agar, a selective and differential medium for the isolation of *Enterobacteriaceae* and *Pseudomonas*. The inoculated XLD plates were then incubated at 35ºC for 24 hours.

Following incubation, 9-10 XLD plates were selected from each barn as a representative sample. Plates were selected based on the presence of individual *E. coli* colonies, appearing on XLD agar as round, white colonies on yellow medium due to

fermentation of the lactose and xylose. For each plate, 100 *E. coli* colonies were collected into 1 mL of sterile LB using sterile toothpicks and vortexed. The *E. coli* suspensions were stored at 4ºC and processed within 7 days.

Colony Pentaplex PCR Assay

To identify the presence of APEC within each cecum, the *E. coli* suspension was subjected to a pentaplex PCR assay, modified from Johnson et al (4). Primers are identified with the expected amplicon sizes in Table 1. The protocol was modified with the following procedure. The Q5 High-Fidelity PCR kit from New England BioSciences was utilized for this study. Master mix was created per reaction that included 10.4 µL DEPC, 4 µL Q5® α Enhancing buffer, 4 µL Q5® α Enhancer, 0.4 µL dNTP (10µM), 0.2 µL Q5® α polymerase, 5 pM of each primer (100 µM; totaling 0.5 µL per reaction), and 0.5 µL of template. Template was created by boiling 50 µL of the *E. coli* suspension for 20 minutes. The template was diluted 1:2 with DEPC prior to addition to the master mix. The PCR cycling was optimized for the Q5 High-Fidelity PCR kit: 98ºC for 30 seconds followed by 35 cycles of 98ºC for 10 seconds, 67ºC for 30 seconds, 72ºC for 15 seconds, and then 72ºC for 5 minutes for terminal extension. The completed PCR product was subjected to 2% agarose gel electrophoresis at 120 mV and visualized under an ultraviolet light. Samples were considered positive for APEC only if all 5 bands were visualized on the gel. Positive and negative APEC controls were utilized. The positive APEC control was grown at 37ºC, subject to colony PCR, purified using the Zymo Research DNA Clean & Concentrator^{TM} -5 Kit, and stored at -20ºC until use due to the instability of the plasmid.

Antibiogram

From the same barn, 24 XLD plates were selected for the antibiotic resistance assay. Isolates were collected into a 96-well round bottom polystyrene plate filled with 300 µL of LB. From each plate, 2 *E. coli* colonies were selected individually using a sterile toothpick and each colony was placed in its own individual well. The 96-well plate was incubated at 37ºC for 18-24 hours and then subjected to pin-replicating into 3 separate 96-well plates containing a different antibiotic media at their respective, predetermined breakpoint concentrations. Antibiotics selected for this assay are ceftiofur (32 ug/mL), enrofloxacin (8 ug/mL), and chloramphenicol (32 ug/mL). Following incubation, the wells were visually analyzed for growth, with any turbidity or breading of colonies considered a positive result.

Statistics

Significance of control versus XPC-treated birds within each source was performed for both prevalence and antibiotic resistance data using a 2-way ANOVA with repeated measures with Tukey's multi-comparison test.

Results

Colony Pentaplex PCR Assay

Thirteen poultry sources were subjected to the APEC survey with approximately 2,000 turkeys and 3,000 chickens tested. All 13 of the poultry sources exhibited a reduction in prevalence of APEC in poultry fed an XPC-based diet (Table 2). Within the control groups of turkeys, the prevalence of APEC positive colonies ranged from 44% to 100%, while in the XPC-treated groups the prevalence ranged from 16% to 50% (Figure 1). Within chickens, the prevalence of APEC positive colonies in control groups ranged from 60% to

93% and in treatment groups ranged from 25% to 50% (Figure 2). The two sources identified as having a non-statistically significant difference in prevalence were C3 and C7. Three sources were identified with a $p < 0.001$ (T4, C2, and C4), four with a $p < 0.005$ (T2, T3, C1, and L1), one with a $p < 0.01$ (C6), and three with a $p < 0.05$ (T1, T5, and C5).

Antibiogram

In this study, *E. coli* colonies collected from poultry ceca were tested for resistance against ceftiofur, enrofloxacin, and chloramphenicol. Ceftiofur was the initial antibiotic tested with results indicating a statistically significant reduction of resistant *E. coli* colonies in poultry fed an XPC-based diet in 11 of the 13 poultry sources (Table 3). The portion of resistant colonies in the control group of turkeys ranged from 55% to 91%, while the XPC-fed group ranged from 30% to 48% (Figure 3A). Chickens from the control group had resistance colonies ranging from 38% to 100% and the treatment group ranging from 17% to 48% (Figure 4A). Most of the poultry sources exhibited a significant p-value less than 0.001, the exception being source C1 with a $p < 0.01$ and T4 and L1, which were not statistically significant.

Enrofloxacin and chloramphenicol resistance assays were included after initial ceftiofur data indicated a reduction in the XPC-fed group. For this reason, enrofloxacin and chloramphenicol resistance data is not included for sources C1-C4. Additionally, no control group resistance data was recorded for these two antibiotics for source T1. Antibiogram data for enrofloxacin revealed 5 of the 8 barns exhibited a statistically significant reduction in resistance (Table 3). Enrofloxacin resistance for turkeys ranged from 30% to 72% in the control group and from 17% to 44% in the XPC-fed group (Figure 3B). Chickens exhibited enrofloxacin resistance ranging from 34% to 73% in the control group and 10% to 23% in the

treatment group (Figure 4B). Sources T2, T3, T5, C5, and C6 had a p-value < 0.001. Of the 8 poultry sources tested for chloramphenicol resistance, all exhibited a statistically significant reduction in chloramphenicol resistant colonies. Chloramphenicol resistance in turkeys ranged from 58% to 99% in the control group and from 20% to 51% in the XPC-fed group (Figure 3C). Chickens in control groups exhibited a resistance range of 85% to 96% and in treatment groups a range of 22% to 52% (Figure 4C). All 8 sources exhibited a significant difference indicated by a p-value < 0.001.

Discussion

Diamond V Original XPC™ is a *Saccharomyces cerevisiae* fermentation product added to feed that has proved quite successful in increasing growth weight of animals and decreasing intestinal colonization of pathogens (24–26, 28). Given the success of XPC in reducing *Salmonella* Typhimurium and *Campylobacter coli* colonization in poultry (24, 25, 28), this study looked at the effects of XPC on APEC colonization in poultry.

Eleven of the 13 poultry sources tested exhibited a statistically significant reduction in the amount of APEC isolated from the ceca of poultry fed a diet with XPC added. Evidence is highly suggestive of a role the microbiota plays in the reduction of food animal pathogens. Consumption of XPC lowers the intestinal pH which may reduce the success of *E. coli* colonization (35, 36). Likely this occurs because at a lower pH short chain fatty acids such as propionic acid and formic acid can reduce an *E. coli* population by 90% without damaging the cell membrane (37). Further, intestinal butyrate increases in animals fed XPC containing products (38). Butyrate is used by enterocytes as a primary energy source and increases the growth of Lactobacillus spp. while decreasing *Enterobacteriaceae* growth (39). In vitro, XPC has been shown to reduce gastrointestinal inflammation (40) and increase microbial

competition and diversity through modulations in the availability of SCFAs (38). This competitive enhancement strategy supports the growth of organisms that can act as a natural competitive exclusion culture against pathogens in the gastrointestinal tract (41). Our study also revealed that poultry fed an XPC-based diet demonstrate a significant reduction in the number of *E. coli* cells resistant to ceftiofur, enrofloxacin, and chloramphenicol, further supporting the usefulness of XPC as a good alternative to antibiotics in poultry. This evidence parallels what is seen in *Salmonella* recovered from XPC fed animals (25).

In summary, this survey provides substantial evidence for the use of Original XPC™ to help reduce the amount of APEC as well as the number of antibiotic resistant microorganisms in the microbiota of chickens and turkeys. Combining these results with the results of other studies, indicates that overall XPC appears to be an alternative to antibiotics in feed for growth promotion and the prevention of diseases. Future work should be considered to elucidate the components of XPC that are acting on APEC to reduce prevalence in the gastrointestinal tract and what mechanisms result in a reduction of antibiotic resistance.

Table 1. Forward and reverse primers used in the pentaplex PCR assay designed by Johnson et al. (4)		
Gene	**Amplicon size (bp)**	**Sequence**
iroN	553	F: AATCCGGCAAAGAGACGAACCGCCT
		R: GTTCGGGCAACCCCTGCTTTGACTTT
ompT	496	F: TCATCCCGGAAGCCTCCCTCACTACTAT
		R: TAGCGTTTGCTGCACTGGCTTCTGATAC
hlyF	450	F: GGCCACAGTCGTTTAGGGTGCTTACC
		R: GGCGGTTTAGGCATTCCGATACTCAG
iss	323	F: CAGCAACCCGAACCACTTGATG
		R: AGCATTGCCAGAGCGGCAGAA
iutA	302	F: GGCTGGACATCATGGGAACTGG
		R: CGTCGGGAACGGGTAGAATCG

Table 2. Prevalence of APEC in ceca from turkeys, (T), broiler chickens (C), and layers (L) fed a diet with (XPC) or without (control) Diamond V Original XPC™. p-values calculated from a 2-way ANOVA with repeated measures with Tukey's multi-comparison test: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.005$ (***), $p < 0.001$ (****), ns = not significant.

Source	**Control (%)**	**XPC (%)**	**p-value**
T1	96.4	48.4	*
T2	89.7	35.3	***
T3	100	38.9	***
T4	71.7	15.9	****
T5	44.4	22.2	*
C1	69.2	26.4	***
C2	87.5	27.3	****
C3	59.6	36.7	ns
C4	84.4	25.5	****
C5	92.9	50	*
C6	70.4	31.6	**
C7	72.2	50	ns
L1	58.3	19.4	***

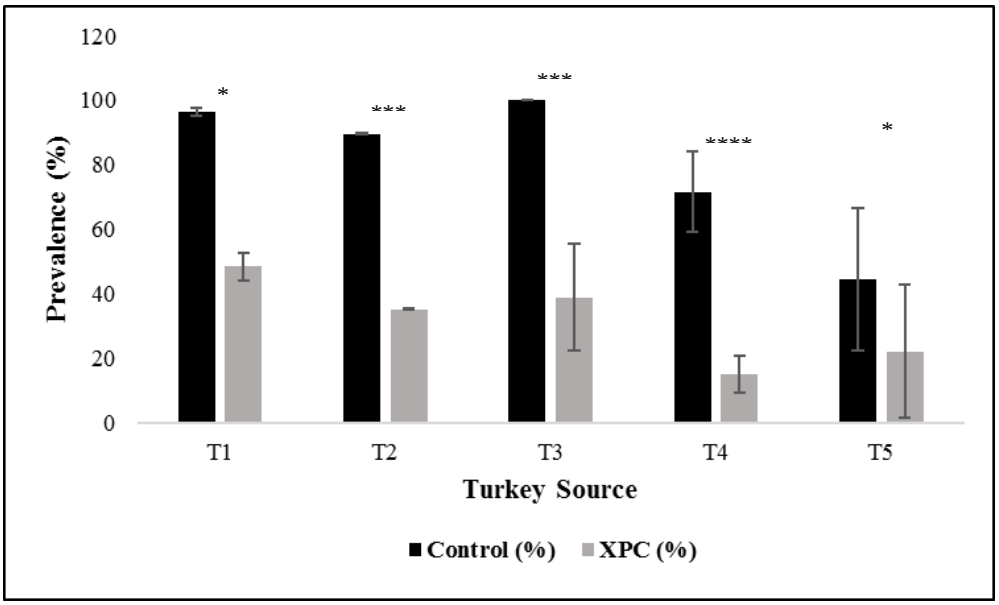

Figure 1. Prevalence of APEC in ceca of ~2,000 turkeys (T) from 5 different sources in the United State. Birds were fed a diet with (XPC) or without (control) Diamond V Original XPC™. p-values calculated from a 2-way ANOVA with repeated measures with Tukey's multi-comparison test: $p < 0.05$ (*), $p < 0.005$ (***), $p < 0.001$ (****).

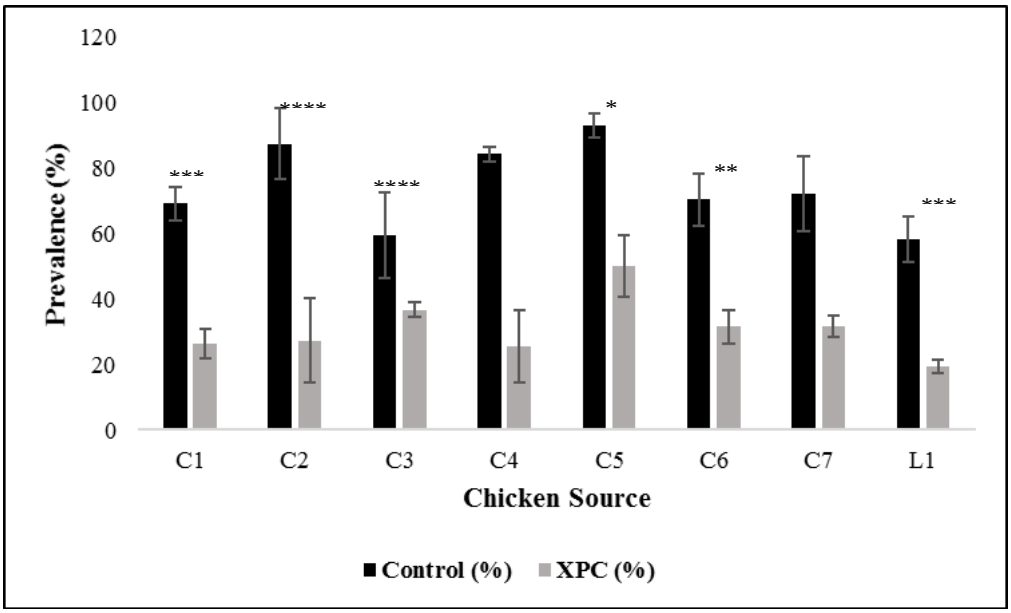

Figure 2. Prevalence of APEC in ceca of ~3,000 broiler chickens (C) and layers (L) from 8 different sources in the United State. Birds were fed a diet with (XPC) or without (control) Diamond V Original XPC™. p-values calculated from a 2-way ANOVA with repeated measures with Tukey's multi-comparison test: $p < 0.05$ (*), $p < 0.001$ (**), $p < 0.005$ (***), $p < 0.001$ (****).

Table 3. Antibiotic resistance rates of E. coli colonies isolated from ceca of turkeys, (T), broiler chickens (C), and layers (L) fed a diet with (XPC) and without (control) Diamond V Original XPC™. p-value calculated from a 2-way ANOVA with repeated measures with Tukey's multi-comparison test: $p < 0.01$ (**), $p < 0.001$ (****), ns = not significant, ND = not determined, N/A = not enough data to calculate.

	Ceftiofur			Enrofloxacin			Chloramphenicol		
Source	**Control (%)**	**XPC (%)**	**p-value**	**Control (%)**	**XPC (%)**	**p-value**	**Control (%)**	**XPC (%)**	**p-value**
T1	82.3	47.9	****	ND	43.8	N/A	ND	42.7	N/A
T2	91.1	45.8	****	51	15.2	****	98.9	50.7	****
T3	80.6	39	****	72.2	17.1	****	97.9	20	****
T4	54.7	47.5	ns	36.7	27.5	ns	64.4	39.6	****
T5	64.6	29.2	****	29.2	5.2	****	58.3	24	****
C1	38	17.4	**	ND	ND	N/A	ND	ND	N/A
C2	65.9	35.4	****	ND	ND	N/A	ND	ND	N/A
C3	75.2	30.8	****	ND	ND	N/A	ND	ND	N/A
C4	58.3	19.8	****	ND	ND	N/A	ND	ND	N/A
C5	100	27.1	****	34	10.4	****	93.1	21.5	****
C6	72.3	25	****	73.4	22.9	****	88.8	30.2	****
C7	69.8	27	****	33.9	24	ns	95.8	26	****
L1	54	48	ns	52	44	ns	85	52	****

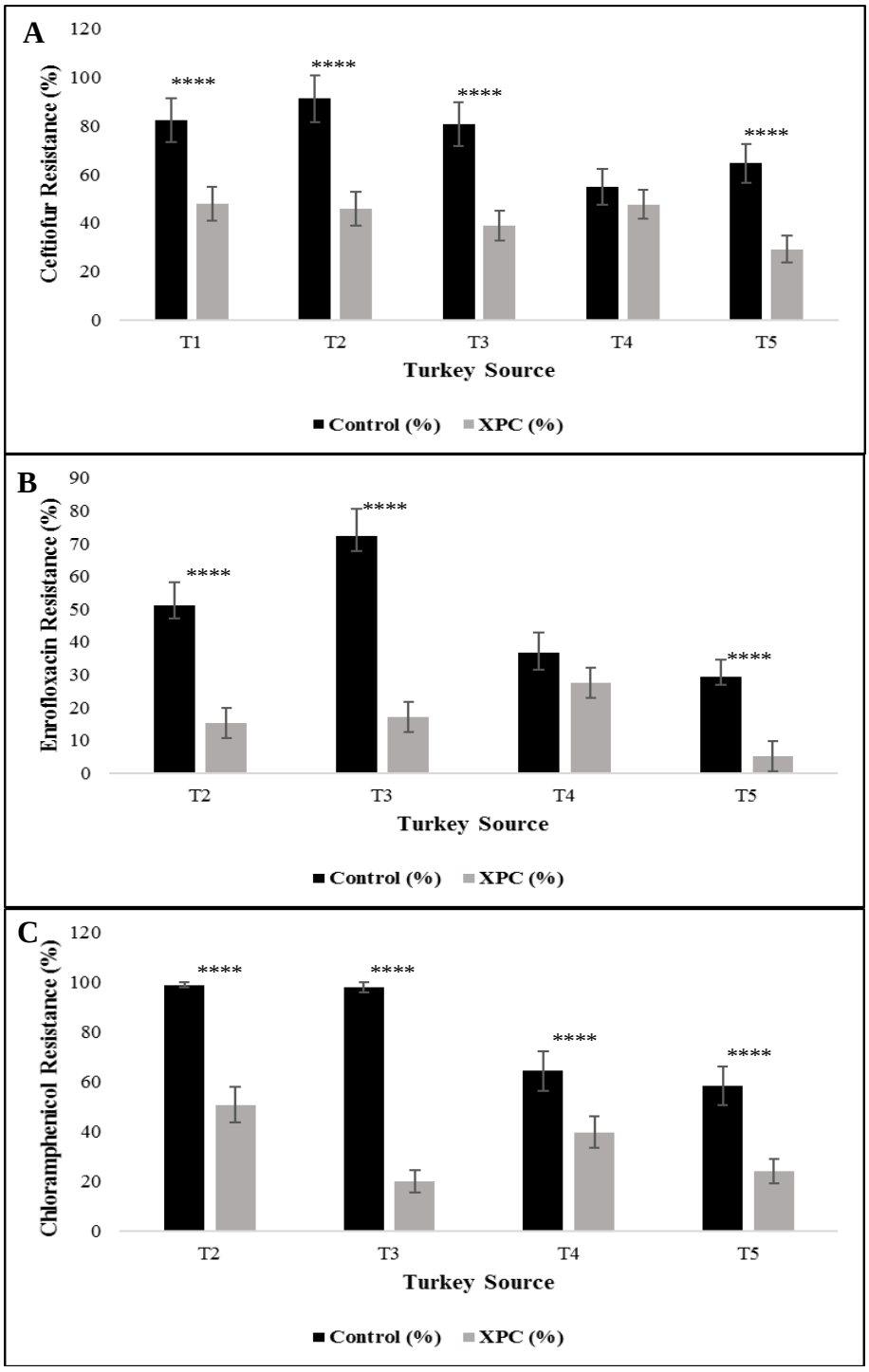

Figure 3. Ceftiofur (A), enrofloxacin (B), and chloramphenicol (C) resistance rates of *E. coli* colonies isolated from the ceca of ~ 2,000 turkeys (T) from 5 sources across the United States. Data collection for source T1 was incomplete for Enrofloxacin and Chloramphenicol resistance. Birds were fed a diet with (XPC) or without (control) Diamond V Original XPC™. p-value calculated from a 2-way ANOVA with repeated measures with Tukey's multi-comparison test: $p < 0.001$ (****).

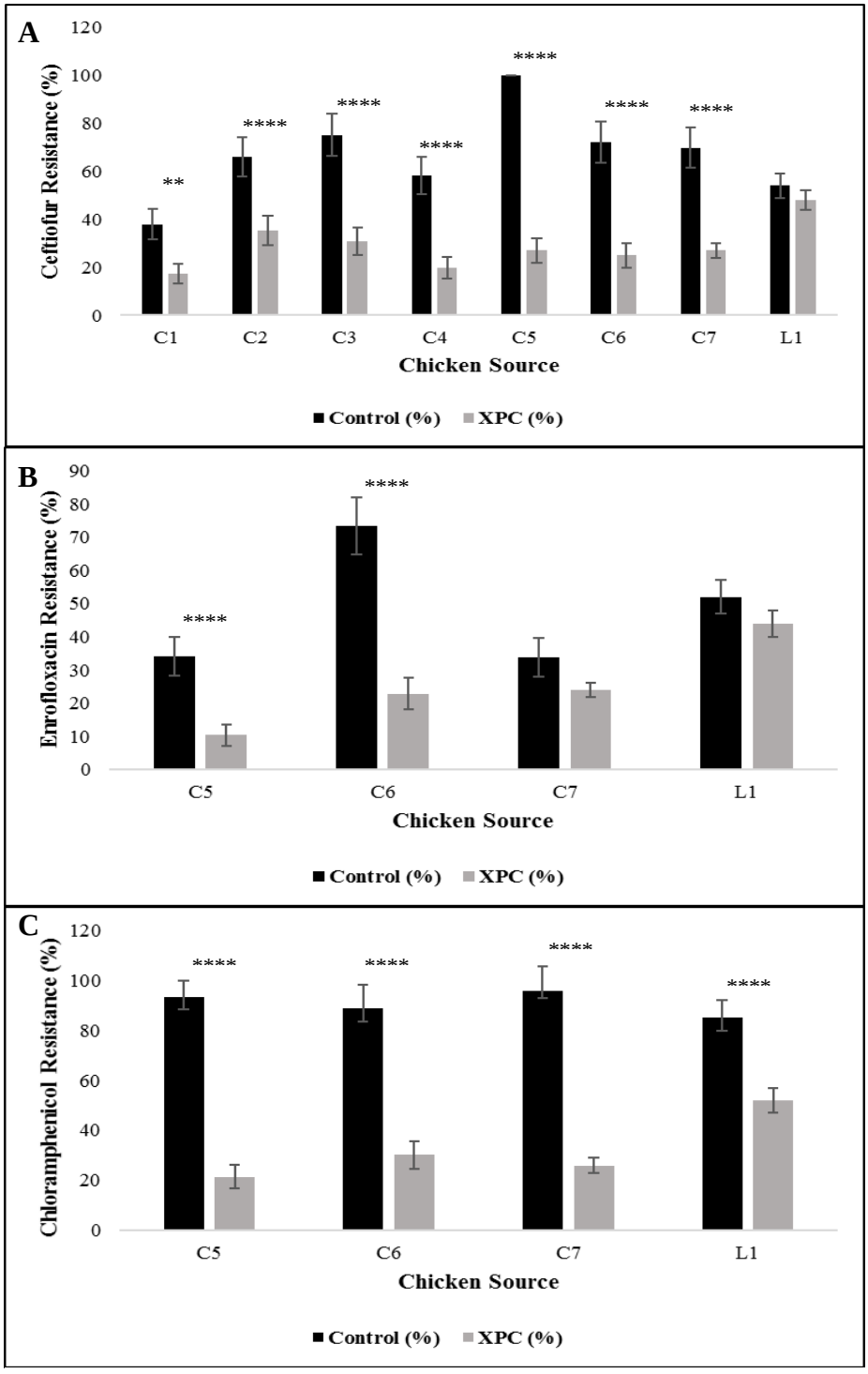

Figure 4. Ceftiofur (A), enrofloxacin (B), and chloramphenicol (C) resistance rates of *E. coli* colonies isolated from the ceca of ~3,000 broiler chickens (C) and layers (L) from 8 sources across the United States. Data collection for source C1-C4 was incomplete for Enrofloxacin and Chloramphenicol resistance. Birds were fed a diet with (XPC) or without (control) Diamond V Original XPC™. p-value calculated from a 2-way ANOVA with repeated measures with Tukey's multi-comparison test: $p < 0.01$ (**), $p < 0.001$ (****).

References

1. **Dziva F**, **Stevens MP**. 2008. Colibacillosis in poultry: unravelling the molecular basis of virulence of avian pathogenic *Escherichia coli* in their natural hosts. Avian Pathol **37**:355–66.

2. **Leitner AG**, **Heller ED**. 1992. Colonization of *Escherichia coli* in young turkeys and chickens. Avian Dis **36**:211–220.

3. **Hicks SJ**, **Rowbury R**. 1986. Virulence plasmid-associated adhesion of *Escherichia coli* and its significance for chlorine resistance. J Appl Bacteriol **61**:209–218.

4. **Johnson TJ**, **Wannemuehler Y**, **Doetkott C**, **Johnson SJ**, **Rosenberger SC**, **Nolan LK**. 2008. Identification of minimal predictors of avian pathogenic *Escherichia coli* virulence for use as a rapid diagnostic tool. J Clin Microbiol **46**:3987–3996.

5. **Dho-moulin M**, **Fairbrother JM**. 1999. Avian pathogenic *Escherichia coli* (APEC). Vet Res **30**:299–316.

6. **Johnson JR**, **Russo TA**. 2005. Molecular epidemiology of extraintestinal pathogenic (uropathogenic) *Escherichia coli*. Int J Med Microbiol **295**:383–404.

7. **Peigne C**, **Bidet P**, **Mahjoub-Messai F**, **Plainvert C**, **Barbe V**, **Médigue C**, **Frapy E**, **Nassif X**, **Denamur E**, **Bingen E**, **Bonacorsi S**. 2009. The plasmid of *Escherichia coli* strain S88 (O45:K1:H7) that causes neonatal meningitis is closely related to avian pathogenic *E. coli* plasmids and is associated with high-level bacteremia in a neonatal rat meningitis model. Infect Immun **77**:2272–2284.

8. **Nicholson BA**, **West AC**, **Mangiamele P**, **Barbieri N**, **Wannemuehler Y**, **Nolan LK**, **Logue CM**, **Li G**. 2016. Genetic characterization of ExPEC-like virulence plasmids among a subset of NMEC. PLoS One **11**:1–16.

9. **Foxman B**, **Brown P**. 2003. Epidemiology of urinary tract infections. Infect Dis Clin North Am **17**:227–241.

10. **Bonacorsi S**, **Bingen E**. 2005. Molecular epidemiology of *Escherichia coli* causing neonatal meningitis. Int J Med Microbiol **295**:373–381.

11. **Zhao L**, **Gao S**, **Huan H**, **Xu X**, **Zhu X**, **Yang W**, **Gao Q**, **Liu X**. 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 **155**:1634–1644.

12. **Tivendale KA**, **Logue CM**, **Kariyawasam S**, **Jordan D**, **Hussein A**, **Li G**, **Wannemuehler Y**, **Nolan LK**. 2010. Avian-pathogenic *Escherichia coli* strains are similar to neonatal meningitis *E. coli* strains and are able to cause meningitis in the rat model of human disease. Infect Immun **78**:3412–3419.

13. **Skyberg JA**, **Johnson TJ**, **Johnson JR**, **Clabots C**, **Logue CM**, **Nolan LK**. 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. Infect Immun **74**:6287–6292.

14. **Waters VL**, **Crosa JH**. 1991. Colicin V virulence plasmids. Microbiol Rev **55**:437–50.

15. **Sherratt DJ**. 1974. Plasmids review. Cell **3**:189–195.

16. **Johnson TJ**, **Thorsness JL**, **Anderson CP**, **Lynne AM**, **Foley SL**, **Han J**, **Fricke WF**, **Mcdermott PF**, **White DG**, **Khatri M**, **Stell AL**, **Flores C**, **Singer RS**. 2010. Horizontal gene transfer of a colV plasmid has resulted in a dominant avian clonal type of *Salmonella* enterica serovar kentucky. PLoS One **5**:1–10.

17. **Mellata M**. 2013. Human and avian extraintestinal pathogenic *Escherichia coli*: Infections, zoonotic risks, and antibiotic resistance trends. Foodborne Pathog Dis **10**:916–32.

18. **Pitout JD**, **Laupland KB**. 2008. Extended-spectrum beta-lactamase-producing *Enterobacteriaceae*: An emerging public-health concern. Lancet Infect Dis **8**:159–166.

19. **Giufrè 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. J Antimicrob Chemother **67**:860–867.

20. **Peirano G**, **Schreckenberger PC**, **Pitout JDD**. 2011. Characteristics of NDM-1-producing *Escherichia coli* isolates that belong to the successful and virulent clone ST131. Antimicrob Agents Chemother **55**:2986–2988.

21. **Johnson JR**, **Johnston B**, **Clabots C**, **Kuskowski MA**, **Castanheira M**. 2010. *Escherichia coli* sequence type ST131 as the major cause of serious multidrug-resistant *E. coli* infections in the United States. Clin Infect Dis **51**:286–294.

22. **Barbieri NL**, **Nielsen DW**, **Wannemuehler Y**, **Cavender T**, **Hussein A**, **Yan S**, **Nolan LK**, **Logue CM**. 2017. mcr-1 identified in Avian Pathogenic *Escherichia coli* (APEC). PLoS One **12**:e0172997.

23. **Davies J**, **Davies D**. 2010. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev **74**:417–433.

24. **Rubinelli P**, **Roto S**, **Kim SA**, **Park SH**, **Pavlidis HO**, **McIntyre D**, **Ricke SC**. 2016. Reduction of *Salmonella* Typhimurium by fermentation metabolites of Diamond V Original XPC in an in vitro anaerobic mixed chicken cecal culture. Front Vet Sci **3(83)**:1-7.

25. **Feye KM**, **Anderson KL**, **Scott MF**, **McIntyre DR**, **Carlson SA**. 2016. Inhibition of the virulence, antibiotic resistance, and fecal shedding of multiple antibiotic-resistant *Salmonella* Typhimurium in broilers fed Original XPC^{TM}. Poult Sci 2902–2910.

26. **Price KL**, **Totty HR**, **Lee HB**, **Utt MD**, **Fitzner GE**, **Yoon I**, **Ponder MA**, **Escobar J**. 2010. Use of *Saccharomyces cerevisiae* fermentation product on growth performance and microbiota of weaned pigs during *Salmonella* infection. J Anim Sci **88**:3896–3908.

27. **Kim SW**, **Brandherm M**, **Freeland M**, **Newton B**, **Cook D**, **Yoon I**. 2008. Effects of yeast culture supplementation to gestation and lactation diets on growth of nursing piglets. Asian-Australasian J Anim Sci **21**:1011–1014.

28. **Hofacre CL**, **Mathis GF**, **Mcintyre DR**, **Broomhead JP**. 2015. Effects of original XPC on cecal colonization by *Campylobacter coli* in broiler chickens: Preliminary report. PO **225**:1–8.

29. **Fukushima M**, **Kakinuma K**, **Kawaguchi R**. 2002. Phylogenetic analysis of *Salmonella*, *Shigella*, and *Escherichia coli* strains on the basis of the gyrB gene sequence. J Clin Microbiol **40**:2779–2785.

30. **Fischbach M a**, **Lin H**, **Liu DR**, **Walsh CT**. 2006. How pathogenic bacteria evade mammalian sabotage in the battle for iron. Nat Chem Biol **2**:132–138.

31. **Johnson TJ**, **Siek KE**, **Johnson SJ**, **Nolan LK**. 2006. DNA Sequence of a ColV plasmid and prevalence of selected plasmid-encoded virulence genes among avian *Escherichia coli* strains. J Bacteriol **188**:745–758.

32. **Murase K**, **Martin P**, **Porcheron G**, **Houle S**, **Helloin E**, **Penary M**, **Nougayrede JP**, **Dozois CM**, **Hayashi T**, **Oswald E**. 2016. HlyF produced by extraintestinal pathogenic *Escherichia coli* is a virulence factor that regulates outer membrane vesicle biogenesis. J Infect Dis **212**:856–865.

33. **Horne SM**, **Pfaff-McDonough SJ**, **Giddings CW**, **Nolan LK**. 2000. Cloning and sequencing of the iss gene from a virulent avian *Escherichia coli*. Avian Dis **44**:179–184.

34. **Gross R**, **Engelbrecht F**, **Braun V**. 1984. Genetic and biochemical characterization of the aerobactin synthesis operon on pColV. Mol Gen Genet **196**:74–80.

35. **den Besten G**, **van Eunen K**, **Groen AK**, **Venema K**, **Reijngoud DJ**, **Bakker BM**. 2013. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res **54**:2325–2340.

36. **Duncan SH**, **Louis P**, **Thomson JM**, **Flint HJ**. 2009. The role of pH in determining the species composition of the human colonic microbiota. Environ Microbiol **11**:2112–2122.

37. **Cherrington CA**, **Hinton M**, **Pearson GR**, **Chopra I**. 1991. Short-chain organic acids at pH 5.0 kill *Escherichia coli* and *Salmonella* spp. without causing membrane perturbation. J Appl Bacteriol **70**:161–165.

38. **Possemiers S**, **Pinheiro I**, **Verhelst A**, **Van Den Abbeele P**, **Maignien L**, **Laukens D**, **Reeves SG**, **Robinson LE**, **Raas T**, **Schneider YJ**, **Van De Wiele T**, **Marzorati M**. 2013. A dried yeast fermentate selectively modulates both the luminal and mucosal gut microbiota and protects against inflammation, as studied in an integrated in vitro approach. J Agric Food Chem **61**:9380–9392.

39. **Gantois I**, **Ducatelle R**, **Pasmans F**, **Haesebrouck F**, **Thompson A**, **Hinton JC**, **Immerseel F Van**, **Hautefort I**. 2006. Butyrate specifically down-regulates *Salmonella* pathogenicity island 1 gene expression. Appl Environ Microbiol **72(1)**:946–949.

40. **Jensen GS**, **Hart AN**, **Schauss AG**. 2007. An antiinflammatory immunogen from yeast culture induces activation and alters chemokine receptor expression on human natural killer cells and B lymphocytes in vitro. Nutr Res **27**:327–335.

41. **Callaway TR**, **Edrington TS**, **Anderson RC**, **Harvey RB**, **Genovese KJ**, **Kennedy CN**, **Venn DW**, **Nisbet DJ**. 2008. Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. Anim Heal Res Rev **9**:217–225.

CHAPTER 4: GENERAL CONCLUSIONS

With the advent of further antibiotic usage restrictions in the Veterinary Feed Directive and the rising concern of APEC as a zoonotic pathogen, research identifying regulatory methods for APEC in poultry is critical. Demonstration of the public health concern of APEC was exemplified by the increase in antibiotic resistance and high transmission of virulence plasmids. A combined concern for poultry and human health led to a desire to identify a prophylactic treatment for APEC to mitigate disease in poultry and the propagation of the pathogen via food sources. The survey presented in this thesis successfully analyzed poultry from sources across the United States for APEC prevalence and *E. coli* antibiotic resistance. Results revealed that Original XPC^{TM} is efficacious in reducing poultry carriage of APEC and reducing the presence of *E. coli* resistant to ceftiofur, enrofloxacin, and chloramphenicol. Identification of a prophylactic treatment for APEC is economically beneficial for the poultry industry and will potentially reduce the threat to public health.

Since this was a surveillance study, much more work needs to be done to elucidate the mechanisms of action of XPC. Initial studies should be done to determine if there is a reduction in virulence, identified by the loss of function of any of the important virulence genes. Genetic regulation of the virulence or resistance genes by a component of XPC or the gut microbiota could be a point of mechanistic action. Additionally, further studies should be done evaluating the effects of XPC on the overall gut microbiota, including but not limited to community sequencing and calculating concentration changes of metabolites in the GI tract. Finally, additional surveillance studies should be done testing the efficacy of XPC on other *Enterobacteriaceae* pathogens and viruses across numerous animal species.