PREVALENCE OF EXTENDED -SPECTRUM BETA-LACTAMASE PRODUCING COLIFORM BACTERIA IN FED CATTLE RECEIVING METAPHYLAXIS

by

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ABSTRACT

The use of antimicrobials – and antibiotics in particular – in livestock production for prevention, control and treatment of bacterial diseases has expanded significantly over the decades. Bacterial resistance to any particular antibiotic can be a natural property of the bacteria, or else acquired as a gene-based mechanism. A field trial was conducted to evaluate the long-term effects of two metaphylactic antibiotic regimens on extended-spectrum beta-lactamase (ESBL) producing fecal *E. coli* in feedlot cattle. A total of one hundred thirty-four crossbred beef steers (initial BW = 390 ± 18.4kg) were used in a randomized complete block design. Upon arrival (d -3), cattle from two sources received routine processing and their body weights were recorded. Steers were assigned randomly to treatment pens (11 to 12 head per pen; 12 pens total) with 1 to 2 steers randomly selected to be a sentinel control and receive no treatment to evaluate antimicrobial resistance among treatments within the pen. Each of the 3 treatments was replicated 4 times. The trial began on d 0 with the initial fecal sample collected and animals receiving the previously assigned 1 of 3 treatments consisting of: 1) control, no antimicrobial administered; 2) ceftiofur (Excede®, Zoetis, Kalamazoo, MI) administered 6.6 mg/kg BW in the caudal aspect of the left ear; or 3) tulathromycin (Draxxin®, Zoetis) administered 2.5 mg/kg BW subcutaneously in the neck. Subsequent fecal samples (n=804) were collected on d X, Y, Z… to determine presence of ESBL from *E. coli*

bacteria and BW was obtained simultaneously to determine performance. There was no difference in health (data not shown) or performance ($P \geq 0.10$) data observed in this study; however, there was a tendency for ADG to be reduced from d 14 to 28 in the ceftiofur-treated cattle ($P = 0.10$). No differences in carcass traits were observed ($P \geq 0.43$). Extended spectrum ß-lactamase and AmpC bacteria were prevalent in the fecal samples across all days and treatment groups. There was no difference (P = XX) between treatment groups across days in terms of sample-level prevalence of ESBL or AmpC *E. coli*. A difference (P = XX) was observed, however, in the bacterial level prevalence and quantity of bacteria growing on MacConkey plus ceftriaxone (4µg/ml: MAC-CEF) plates only on d 7 for the ceftiofur treated group, when total coliform counts also were significantly reduced on plain MAC plates. On d 14 we reported that coliform counts had returned to baseline prevalencebefore antibiotic administration. At the time of harvest, no differences were detected among treatment groups. Among all fecal samples, 143 bacterial isolates exhibiting the ESBL/AmpC phenotype also exhibited resistance to as many as 9 classes and 13 individual antibiotics. Among these, 42 isolates were of the true ESBL phenotype while 101 were of the more common AmpC phenotype commonly found in North American cattle populations.

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CHAPTER I

REVIEW OF LITERATURE

Introduction to Antimicrobial Resistance

Antimicrobial resistance is a challenge that concerns both public health and economic prosperity worldwide. The efficiency of antimicrobials is being put to the test in comparison to effectiveness, and the beneficial aspect in regards to the economic market. Ongoing research to develop new and improved antibiotic as well at the financial efficiency of the pharmaceutical market is constantly in action to ensure public and animal health and safety (O'Neill, 2015). Understanding the role of commensal and pathogenic bacteria and their capabilities of becoming resistant to antimicrobials is a complex process that challenges the food producing industry and both human and animal health. Antimicrobial use in food animals has been associated with antimicrobial resistant bacterial pathogens found in humans (Angulo et al., 2004).

Resistance in *Methicillin-Resistant Staphylococcus aureus* (MRSA) is a major concern in human medicine, and is known for severe infections, and some cases of death. Data suggest that food animals in the United States could become an important reservoir for this bacterium; further, it estimated that 1.5% of the United States population is colonized with MRSA (Smith, 2009). In a paper published in 2007, the authors presented statistics on infections from MRSA leading to death in the United States suggesting they have overcome other infectious diseases, such as HIV/AIDS, with 94,000 infection and greater than 18,000 deaths in 2005 (Kleven, 2008). Research (Smith, 2009) was

conducted to examine the prevalence of MRSA in swine and swine workers in two swine farming production systems in Iowa and Illinois. Twenty-four samples from each location were collected from weaned pigs between 6 and 18 weeks old, and all workers willing to participate on 45 farms in five major swine producing states in the USA that were readily accessible to the collaborating groups: University of Iowa, The Ohio State University and University of Minnesota. Samples were collected over a two-year period starting in April of 2008 and ending in July of 2010. The study was designed so that half of the farms sampled by each collaborating group would be conventional confinement farms and half would be farms raising pigs without use of antibiotics (antibiotic-free, ABF). Farms were selected by convenience, based on participation of the producers. However, due to difficulty in recruiting eligible farms, ultimately 45 farms were included in the study: 18 farms in Minnesota (9 conventional, 9 ABF); 18 farms in Iowa-Illinois (9 conventional, 9 ABF); and 9 farms in Ohio-North Carolina (6 conventional; 3 ABF). After giving written informed consent, participants completed a questionnaire providing demographic data and provided swab samples from the nares and oropharynx to determine MRSA colonization status. The survey recorded data on potential risk factors for MRSA colonization, including information about contact with swine and use of personal protective equipment. All pigs sampled on the 21 ABF herds were negative for MRSA. The MRSA prevalence among pigs on conventional farms overall was 8.5%. On MRSA-positive farms, the prevalence of positive cultures ranged from 17% to 100% of weaned pigs sampled.

Four of the participants (2.7%) reported a skin or soft tissue infection within the prior year, whereas none reported previous diagnosis with a MRSA infection. A total of

31 of 148 farm workers (20.9%) were culture positive for MRSA. Of these, 87% worked on farms where MRSA was detected among the sampled swine. Seventy-seven percent of the samples that tested positive came from 40 workers on the two farms with the highest prevalence of MRSA in pigs. It was concluded that working on a farm with a MRSA-positive herd was by far the greatest risk factor for worker colonization (Wardyn et al, 2015).

Resistance may emerge or expand when bacterial subpopulations with pre-existing resistance or reduced susceptibility are selected for in the presence of antimicrobial pressure. This selected subpopulation of microbes can then more readily pass on the specific resistance genes, such as through replication where plasmids transfer the resistance genes from one organism to another (Ribble et al., 2010). Evidence has shown that it is common for a natural resistance to occur with specific antibiotics that bacteria regularly interact directly with in the environment (e.g., soils and soil bacteria, from which many of our antibiotic classes arose. By definition, antibiotic resistance reflects the ability of a microorganism to withstand the effects of an antibiotic. Antibiotic resistance may further evolve through any of 3 mechanisms: 1) naturally through resistance *a priori* found in certain types of bacteria and then shared with other bacteria; 2) via random genetic mutations; or 3) synthetically engineered (anthropogenic) by applying evolutionary stress on a population of specific bacteria to further study the resistance.

The community of resistance genes in the environment is due to a mix of naturally occurring resistance and those present in animal and human waste and the selective effects of bacteria. Antibiotic resistance may also be a consequence of evolution via

natural selection of randomly occurring mutations. The antibiotic action is an environmental pressure; those bacteria that have a mutation allowing them to survive exposure to a particular antibiotic will live on and continue to reproduce their line. This is also known as clonal expansion. They will then pass resistance traits to their offspring, which develop into a fully resistant generation (Molbak, 2004). There is evidence that natural occurring resistance is common. However, the use of antibiotics in human and veterinary medicine for prophylactic and treatment purposes has led to a more rapid increase in antibiotic resistance and an increase of antibiotic resistant genes in the horizontal gene pool than would likely occur in nature (Bakry, 2014).

The introduction of antimicrobial agents in human clinical medicine and animal agriculture has been one of the most important breakthroughs of the 20th century (Chadwick et al., 1997). However, resistance was likely promoted shortly after the introduction of antimicrobial use and in all known cases of antimicrobial discovery the emergence of antimicrobial resistance has followed a similar pattern of resistance; that of resistance to specific drugs by specific bacteria (Levy, 1982). It has now become clear that antimicrobial resistance poses a threat to the economics of production, the pharmaceutical industry, and most importantly human and animal health (WHO, 2001).

Emergence of Multi-Drug Resistance

The global increase in the prevalence of multidrug–resistant (MDR) bacteria has posed a threat to physicians' ability to provide appropriate therapy and solutions for infections and illnesses. The relationship between antimicrobial drug concentration and infecting pathogen population reduction is of primary interest (Levy, 1992). Data arising from Jumbe and others (2003) involved mice infected with the bacterium, *Pseudomonas*

aeruginosa, and treated with a fluoroquinolone antibiotic. A mathematical model was developed that defined the relationships between antimicrobial drug exposure and changes in drug-susceptible and drug-resistant bacterial subpopulations at an infection site. Dosing regimens and consequent drug exposures that amplify or suppress the emergence of resistant bacterial subpopulations were identified and prospectively validated. Resistant clones selected *in vivo* by suboptimal regimens were characterized. While there were no mutations identified with the quinolone resistance, all resistant clones confirmed efflux pump overexpression; that is, expressed as trying to move antibiotic compounds out of the bacterial cell. The study altered a standard mouse-thigh bacterial infection model to observe the effect of increasing doses of an antibiotic on the *in vivo* amplification and suppression of the drug-susceptible and -resistant populations over 24 hours. To examine the inoculum effects of the *P. aeruginosa* test strain on dose-response relationships, a single dose of levofloxacin was administered. Dose-ranging studies were performed with mice infected with significant organisms in both posterior thigh muscles.

The mutational frequency of resistance of the challenge strain explained the differences in antimicrobial response with different inoculates. Drug-resistant *P. aeruginosa* mutants were quickly isolated from treated and control mice infected with the higher inoculum. With such rational dosing-regimen designs, one can maintain the sensitivity of the infecting flora to the drug, in return benefiting future cases that are in need of treatment. Horizontal transmission of genes among bacteria is minimized, largely because the first-stage mutants are inhibited. This is simply done by detecting the resistance-inhibiting drug-exposure target and using population pharmacokinetic

information to evaluate other options of therapeutic antibiotic doses for their ability to achieve the drug-exposure target above the already achieved MIC values recorded in this study (Jumbe et al., 2003).

Treatment administrations and drug exposures that fluctuate the development of resistant bacterial subpopulations were acknowledged. The database included 107 acutely ill patients, 128 pathogens, and five antimicrobial regimens. Through concentrations and culturing, antimicrobial pharmacokinetics were distinguished as well as sensitivity tests on tracheal aspirates to examine resistance. Resistant clones selected *in vivo* by suboptimal regimens were also depicted and named in a category. Pharmacodynamic models were illustrated to isolate features associated with the probability of developing bacterial resistance. The probability of achieving resistance-suppression contact in humans, resulted in clinically approved antibiotic dose. The undesirable microbial population considerably exceeded the mutation frequency with the increased infected inoculum. High level of exposures that killed the sensitive populations, the resistant populations survived. Resulting in subpopulation to be selected by the drug pressure. Dosing-regimens are designed in such a way to preserve the sensitivity of the infecting flora to the drug. Due to the first-stage mutants that are inhibited, horizontal gene transmission was minimized (Jumbe et al., 2003).

Bacterial cells may implement more than one mechanism to resist an antibiotic. Co-operation between several resistant mechanisms can also generate higher levels of resistance than any single mechanism. Examples of this would-be efflux and penetration mechanisms with affected antibiotics, such as tetracyclines, that otherwise might exhibit low levels of resistance. By-pass and enzyme detoxification mechanisms could likewise

result in higher levels of resistance with antibiotics such as ß-lactams or macrolides (Blair et al., 2015). Drug pressure is another factor that must be considered. Bacteria can become resistant as a result of genetic mutations; these can be transferred between bacteria and groups of bacteria. Under selective pressure of antibiotic exposure, these strains then proliferate. Different types of antibiotics inhibit growth (bacteriostatic) or kill (bactericidal) bacteria using unique mechanisms. Various classes of antibiotics work on different aspects of bacterial replication. Under selective pressure of antibiotic exposure, these resistant strains then proliferate. Bacteria evolve resistance to antibiotics in response to environmental pressure exerted by the use of antibiotics; further, many of these bacteria are significant pathogens (Hauger, 2004).

The concerning challenge with the use of antibiotics in animals on human health, arises from antimicrobials used in food animals that potentially select for resistant bacterial strains which may subsequently be transferred to humans via direct contact or ingestion of contaminated food or water. Many classes of antibiotics used in food animals have similarities to those used in humans, and these are capable of selecting for specific resistances to antibiotics that are used in human medicine (McCormack, 2012). The majority of antibiotics arise from only a few types of drugs that are considered the main classes of antibiotics. Aminoglycosides are commonly used to battle infections caused by Gram-negative bacteria. Aminoglycosides stop the bacteria from producing proteins. Cephalosporins currently have 5 different types, which are classified by the generation level. Generally, the higher the generation (up to 4^{th} generation), the more Gram-negative antimicrobial properties that are associated with the antibiotic. Generally, later generation cephalosporins have less chance of bacteria becoming resistant, though this is not always

the case. Other modifications to approved variants of cephalosporin are to allow the drug to be in effect for a longer period of time. Fluoroquinolone, is a type of antibiotic active against several different types of bacteria; however, it is mainly used to treat urinary tract infections, respiratory infections, and skin infections. Its mode of action is to inhibit the bacterium's ability to replicate DNA, so it is harder for the bacteria to reproduce. The first type of antibiotic to be discovered, penicillin, is still widely used to treat dental infections, respiratory infections, gonorrhea, urinary tract infections, and skin infections. This class of antibiotics operates by obstructing the growth of bacteria cell wall, and thus is typically only useful for Gram positive infections. Following treatment, the bacterial cell wall becomes porous and decays, and the bacteria are killed by the antibiotic. Tetracyclines are most common used for upper respiratory infections, Lyme disease, mild acne, and typhus. They are used to fight a wide variety of bacterial infections. Doxycycline and minocycline are just two types of tetracycline, older varieties include chlortetracycline and oxytetracycline which are widely used in agriculture. Macrolides are used to treat gastrointestinal tract and other infections. They bind ribosomes in susceptible bacteria which reduces protein production, thereby aiding in the elimination of the harmful bacteria over time (Nikaido, 2009).

Bacteria may acquire and express multiple mechanisms of resistance (Lowrance et al., 2007). Bacteria that are both helpful and harmful are found in the digestive tract of animals and humans; indeed, they are ubiquitous and are always present via consumption of food and water, or through occupational or environmental exposure. Several strategies and solutions have been proposed to try to overcome the issue of antibiotic resistance in humans and livestock (van den Bogaard, and Stobberingh, 1999). Prevention should be

the first solution that is turned to when groups of concerned individuals come together to try and solve sensitive topics such as resistance; prevention methods may include the use of immunomodulators and non-antimicrobial therapies such as vaccination programs, and backgrounding (Ives and Richeson, 2015).

Understanding resistance mechanisms in bacteria is an entire scientific discipline unto itself. It is highly necessary for the comprehension and development of the knowledge, research and tools needed to distinguish infection and illnesses that lead to resistance (Chadwick et al., 1997). Methods are put into play by tests such as a phenotypic testing, and more improved genotypic tests that evaluate the susceptibilities of the microbes to antibiotics (Bergeron and Ouellette, 1998). Considering the type of infections, the rate at which resistant microbes develop, and the targeted organs or tissue of infection are all elements that need to be understood in regards to the development of prevention programs. Action plans are developed with goals focusing on strengthening health care, public health, veterinary medicine, agriculture, food safety, research and manufacturing.

As decades pass, more and more research is becoming available to understand the difficult concept of antimicrobial resistance and to reach a compromise with the disparate and large group of organizations and policy makers that advocate for, and implement regulations for proper use of antibiotics to mitigate resistance. Although the direct quantitative relationship between the amount of antibiotic used and the frequency of resistance is still lacking, this obvious issue may seem simple and easy to reverse and fix; however, it could possibly be the most difficult challenge facing both human medicine and animal health (Harrison and Lederberg, 1998). Clinical problems can develop when

susceptible strains are diminished, permitting the resistant microbial base to expand. This is the most important consideration when using antibiotics to treat infections or control disease in humans or animals. Furthermore, the same antibiotic administered in different ways may have significantly different effects on antibiotic resistance (van den Bogaard and Stobberingh, 1999). When penicillin was given in less than therapeutic doses and for relatively long periods of time (5 days), patients' risk for carriage of penicillin resistant pneumococci escalated (Georgeopapadakou, 1993). Additionally, the dosages of antibiotic being distributed among health care patients and within a specific geographic area can greatly influence the occurrence of antibiotic resistant bacteria.

Antibiotic resistance can also be introduced into a microorganism through transformation, which is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material from its surroundings through the cell membrane. This can be a useful way of implanting exogenous genes into the microorganism. Most antibiotics in human health use are related to organic products and bacteria have co-evolved mechanisms to avoid their inhibitory action (Gow et al., 2008). Antimicrobials are critical to the existing beef production programs that occupy the industry today. A wide variety of antibiotic classes are available for beef cattle, all subjected to Food and Drug Administration (FDA) approval and post-approval compliance. This and other organizations closely monitor animal health and welfare, in response to reports of adverse reactions, and deal with controversial topics such as growth promotion uses of antibiotics (Cameron and McAllister, 2016). Most of the microorganisms found in the intestinal tract consist of massive (i.e., $> 10^{12}$ bacteria per gram of feces) populations of harmless bacteria; however, these microbiomes contain

adaptable pathogens that acquire and further mutate genes that help express resistance to commonly administered antibiotics (Acar and Rostel, 2001). Antibiotics – and, more broadly antimicrobials – are used in beef cattle for therapeutic claims such as treatment, control, and prevention of rapidly spreading, serious bacterial infections and diseases that may cause a large loss of animal health, welfare, and production.

Resistance that is associated with antimicrobials used for BRD treatment is an issue that may have consequences in the cattle industry as well. It is extremely important that producers attempt to decrease unnecessary use of antimicrobials. Such practices that could be utilized to reduce the dependency we currently have on antibiotics include: limiting the over-the-counter availability of antimicrobials such as through the newest Veterinary Feed Directive law (FDA, 2015), assuring optimal colostrum intake at birth, backgrounding, and being aware of clinical signs at an early stage of disease, including younger ages, being aware of health risk status of purchased lots of cattle, and through quarantine of newly introduced animals to a herd (Schneider, 2008). Another key to lowering the use of excessive antibiotic use is the diagnosis of bovine respiratory disease (BRD) of animals as early in infection as possible. This helps to overcome the disease process before it has time to cause unrepairable damage to the animals' immune system or vital organ functions (e.g., lungs) and also can help prevent further spread causing an epidemic outbreak within the population.

Over the last 15 years, decreases have been noticed in the susceptibility of *Mannheimia haemolytica* to danofloxacin, tilmicosin, tulathromycin, enrofloxacin, and florfenicol, with the latter two antimicrobials, in particular, associated with increased resistance in *Histophilus somni* (Portis et al., 2012). Further, the minimum inhibitory

concentrations (MICs) of tetracycline, tilmicosin, and tulathromycin have also been increasing for *H. somni* (Kilma et al., 2014). A third BRD bacterial pathogen, bovine-sourced *Pasteurella multocida,* has also shown a significant decrease in susceptibility to florfenicol, spectinomycin, tetracycline, tilmicosin, and trimethoprim-sulfamethoxazole (Kilma et al., 2014).

The emergence of resistant microorganisms is caused either by mutations in existing genes or else the acquisition of mobile genetic elements carrying resistance genes. Exposure to antibiotics is what provides the crucial selective pressure needed for the propagation and spread of resistant pathogens (Roca et al., 2015). The use, overuse, and misuse of antibacterial agents in both human and veterinary medicine, is the driving incubator of the increasing rates of resistance. The spread of resistant bacteria from livestock to humans can take place via a number of routes, with food-borne infections perhaps being the most concerning with enteric bacterial pathogens such as *Salmonella enterica, Campylobacter coli/jejuni*. On the other hand, for other resistant pathogens such as *MRSA,* a direct link between livestock and human transmission is unlikely to be via the food supply; rather, occupational (direct contact) risks are well established, but do not tend to extend beyond the veterinarian, farmer, farm worker and their families. Bacteria – and even antibiotic residues – from food producing animals are spread throughout the environment, mostly by waste materials such as manure or compost (Roca et al., 2015). The need to reduce the overall use of antimicrobials in agriculture and human medicine is a topic that has been extensively discussed, and recent experiences from several European countries suggest that major reductions can be achieved without significant negative effects (Grave et al., 2010) on animal health or

productivity; ideally, in the longer term with benefits for public, environmental, and animal health.

Bovine Respiratory Disease

Bovine respiratory disease (BRD) is the leading cause of morbidity and mortality in North American beef cattle (Watts and Sweeny, 2010). The primary bacterial pathogens associated with BRD are *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni*. *M. haemolytica* is considered the most concerning of the bacteria. These bacteria reside in the nasopharynx of cattle and develop into respiratory infections in the lungs of cattle that have been subjected to a variety of stresses. Factors that permit adherence to and proliferation in the lungs and factors that cause tissue damage and inflammation are thus known to be the main factors promoting pathogenesis. Bacterial factors include adhesion proteins, capsular polysaccharides, outer membrane proteins, iron-binding proteins, lipopolysaccharides, enzymes and toxins. These bacterial products function to evade and damage the immune system, create a severe inflammatory response (Confer, 2009). All of the bacterial pathogens considered important in BRD can be isolated from the upper respiratory tract of healthy cattle and are readily identified in the nasopharynx of cattle in most populations (Callan and Garry, 2002). The viral pathogens associated with BRD are also universal; however, commingling cattle from multiple sources may increase exposure to antigenically heterogeneous pathogens. To control the health challenge of BRD in the cattle industry, antibacterial therapy is highly important. A large selection of antimicrobial agents have been approved for the treatment of BRD, including: oxy- and chlor-tetracycline, ceftiofur, tilmicosin, tulathromycin, florfenicol, enrofloxacin, danofloxacin and several others (Watts and Sweeny, 2010).

Metaphylaxis

Mass medication, also known as “prophylaxis” or “metaphylaxis”, is a common prevention or control method, respectively, given to cattle upon arrival to reduce the number of BRD cases (Lofgreen, 1983; Nickel and White, 2010). Involvement of bacterial pathogens in BRD (versus imply viral pathogens) explains why mass treatment of high risk cattle effectively reduces both morbidity and mortality in multiple controlled trials. Such mass treatment can be accomplished in one of three ways: 1) parenterally (i.e., by injection), 2) in feed, or 3) in water. Administration of parenteral products to calves that are at very high risk for BRD – or in pens experiencing an outbreak – (metaphylaxis) has consistently been found to reduce morbidity. Some of the more popular antimicrobials found to be effective in metaphylaxis include: ceftiofur crystalline free acid, florfenicol, tilmicosin, tulathromycin, danofloxacin, enrofloxacin, chlortetracycline, oxytetracycline, and sulfamethazine (Ives and Richeson, 2015; Taylor et al., 2010). In addition to reduced morbidity and mortality, treated calves also exhibit better average daily gain, feed efficiency, and overall performance. These factors support the use of metaphylaxis and have proven to be very cost-effective. Metaphylaxis is the treatment of a population or group of cattle with an FDA approved antimicrobial with the intent of the controlling the incidence of acute-onset disease of newly received, highly stressed cattle (Ives and Richeson, 2015). Post-arrival metaphylaxis has been shown to reduce disease rates by 50% and to reduce mortality by 25% (Urban-Chmiel and Grooms, 2012). However, as described previously, antibiotic use increases the risk of targeted and non-targeted bacteria developing resistance; therefore, it is necessary to use antibiotics judiciously. Other studies have suggested that metaphylaxis used in conjunction with an

on-arrival immunization program is especially beneficial for reducing BRD occurrence in newly received calves (Thompson and White, 2006). The positive effect of metaphylaxis is observed in the case of reduced morbidity and mortality, but also because of significantly increased average daily gain and feed efficiency. Establishing BRD prevention and control systems benefits producers by reducing economic loss and reducing disease incidence, the latter of which has both food safety and quality implications (Urban-Chmiel and Grooms, 2012).

Consumer Perceptions of Antibiotic Use and Resistance in Livestock

The use of antimicrobial compounds in food animal production provides demonstrated benefits, including improved animal health, higher production and, in some cases, reduction in foodborne pathogens (Larson and Pierce, 2001). However, use of antibiotics for agricultural purposes, particularly for growth enhancement, has been increasingly scrutinized by the general public, as it is perceived to contribute to the increased prevalence of antibiotic-resistant bacteria of human significance. The transfer of antibiotic resistance genes and selection for resistant bacteria can occur through a variety of mechanisms, which may not always be linked to specific antibiotic use (Marshall and Levy, 2011). Prevalence data may provide some perspective on occurrence and changes in resistance over time; however, the reasons are diverse and complex. Much consideration has been given to this issue on both domestic and international fronts, and various countries have enacted or are considering tighter restrictions or bans on some types of antibiotic use in food animal production (Marshall and Levy, 2011). In some cases, banning the use of growth-promoting antibiotics appears to have resulted in decreases in prevalence of some drug resistant bacteria; however, subsequent increases in

animal morbidity and mortality, particularly in young animals, have resulted in greater use of therapeutic antibiotics that are often from antimicrobial classes of greater relevance to human medicine (Alexander et al., 2008). While it is clear that use of antibiotics can result in significant pools of resistance genes among bacteria, including human pathogens, the risk posed to humans by resistant organisms from farms and livestock has not been clearly defined (CDC, 1999). Livestock producers, animal health experts, the medical community, and government agencies consider effective strategies for control of resistance cases. It is critical that science-based information provide the basis for such considerations, and that the risks, benefits, and feasibility of such strategies are fully considered, so that human and animal health can be maintained while at the same time limiting the risks from antibiotic-resistant bacteria (Mathew et al., 2007). While it is clear that nosocomial infections in human medicine likely have very little to do with veterinary medicine, a majority of the bacterial antimicrobial resistance observed in foodborne illness in people has been assumed to be from antimicrobial use in food animals that results in resistant foodborne pathogens (Mathew et al., 2007).

During the National Institute for Animal Agriculture Conference in 2014, the topic of perception of animal agriculture was discussed, pointing to a great divide where consumers and producers will never settle or agree on the topic. "*Critics of agriculture religiously argue that livestock account for 80% of all antibiotic use in the United States. Supporters of animal agriculture use the argument that all antibiotic use in livestock is under the control and direction of veterinarians. The biological processes through which resistance emerges are not fully understood. Consumers with opposing perspectives can draw different conclusions from the same data, and for consumers, positions regarding*

food safety and medical care are driven more by emotion than by science" (Dr. Richard Raymond, NIAA Conference 2014).

Public Health Risk and Control

In 2001, the World Health Organization set the basis for the establishment of measures toward controlling antibiotic resistance in animals intended for food. In summary, control measures should reduce the emergence and spread of antibiotic-resistant bacteria, improve use of antimicrobials, establish effective surveillance systems, enforce legislation, and encourage the development of new drugs and vaccines (WHO, 2001). Antibiotic-resistant bacteria of animal origin are considered an important contributor to the overall burden of bacterial resistance to antibiotics. Judicious use of antibiotics in animals is required to reduce the emergence, spread, and propagation of bacteria resistant to currently effective antibiotics. The invention of novel drugs or the use of alternatives to antibiotics should also be encouraged. Still, an increased awareness of antimicrobial resistance by the scientific community and by public health, consumer, and agricultural stakeholders is both alarming and promising at the same time. Therefore, a combined international action is needed toward the solution of antimicrobial resistance.

Mechanisms of Antibiotic Resistance

There are two ways in which bacteria may initially become resistant to an antibiotic; resistance may occur from natural or inherent mechanisms or else by genetic mutation and clonal spread. In the second case, the initial transition tom resistance occurs by a genetic mutation and is followed by sharing or acquiring resistance from another bacterium.

Mutations by definition are rare spontaneous changes of bacterial genetic material, and are thought to occur in one in every 10 million cell divisions (Moore et al., 2014). Many, if not most, mutations are either fatal or else insignificant; however, in some cases an advantage is conferred by the mutation, often in the presence of an external selective pressure such as antibiotic use. Different genetic mutations produce different types of resistance. Some mutations enable the bacteria to produce potent chemicals (enzymes) that inactivate antibiotics, while other mutations remove the cellular target that the antibiotic attacks. While porous cell membranes can allow antibiotics into the cell, modified proteins can aid in pumping the antibiotics into extracellular space.

Bacteria can acquire antibiotic resistance genes from other bacteria – of the same or different species – in several ways. Conjugation is the process of simple mating and another way bacteria can transfer genetic material via pili, including genes encoding resistance to antibiotics found on plasmids, from one bacterium to another (Aminov, 2010). Bacteria also have the ability to acquire untouched DNA from their environment through a process called transformation. Third, viruses specific to bacteria, known as phages, can transfer genetic sequences encoding for antimicrobial resistance when they infect new bacterial strains through a process called transduction. Any bacteria that acquire resistance genes, whether by spontaneous mutation or genetic exchange with other bacteria, have the potential to resist more than one antibiotic. Because bacteria can collect multiple resistance traits over time, they can become resistant to many different classes of antibiotics (Aminov, 2010); further, use of any antibiotic to which a bacterium harbors resistance can therefore lead to expansion of other held resistance traits through a process called co-selection.

Gene Transfer

Genes responsible for antibiotic resistance in one species of bacteria can be transferred to another species of bacteria through various mechanisms. The resistance genes may enable a bacterium to produce enzymes that destroy the antibiotics that prevent the drug from reaching its intracellular target to modify the drugs target site (Bakry, 2014). Resistance that existed before the discovery and commercialization of antibiotics was the system by which bacteria co-existed with microbes producing antibiotics in natural environments such as the soil. Resistance to antibiotics can be innate whereby the micro-organism lacks a specific target site for the antibiotic drug, or antibiotic resistance can be acquired by means of changes in the genetic code of maintaining the genes associated with the DNA or receipt of new genetic material encoding for resistance. Replication of resistance genes depends on replication proficient vector molecules of the host cell, such as chromosomal DNA or plasmids, in which they can integrate (Kehrenberg et al., 2001). Vertical transmission of resistance relates to a chromosomal mutation affecting a bacterial cell; thereafter, the clone replicated from this cell will reproduce and spread. Clonal expansion of a single strain (versus its competition) typically can only take place under the advantages provided by antibiotic use (suppressing the competition). Horizontal transmission may occur within – or among different – bacterial species. A resistance gene located on a plasmid can be transmitted horizontally, independently of the resistant clone. Plasmids are the main system that carry resistance from bacterium to bacterium, and are known for transferring more than one marker of resistance. The larger the plasmid, the increased number of mechanisms of

resistance that may be carried against antibiotics, along with other important factors such as those encoding for virulence and adhesion, among others (Acar and Rostel, 2001).

Emergence of Antibiotic Resistance

The strength of innumerable species of commensal bacteria, along with those that cause diseases in human and animal health, to resist the inhibitory actions of antimicrobial agents has become a global concern. Resistance is spread by not only nosocomial (hospital-based) pathogens, but also in several basic community-acquired organisms. The most applicable ways to manage resistant organisms, depend in part on the pathways where resistance has developed. However, these pathways differ greatly from organism to organism, and setting to setting, making it difficult to isolate and manage specific pathways to contain resistance (Tenover and McGowen, 1996). Bacteria from both hospital and community settings are rapidly becoming resistant to a number of conventional antibiotics, which can be thereby be classified as multi-drug resistant (MDR).

Multi-Drug Resistance

Multi-drug resistance is occurs with more than one class of antimicrobial; generally speaking, the term is reserved for bacteria with resistance to greater than 2 classes of antimicrobial. In cases such as MDR, it is common to observe resistance to several classes of antimicrobial drugs in relation to gene encoding and phenotype linking matching genetic elements such as plasmids and integrons (Lowrance et al., 2007). As one example, cattle, pigs, and humans were included in a study that characterized multi-drug resistant *Salmonella* isolates that, most importantly, were resistant to extended-spectrum cephalosporins; further, it was shown that a majority of resistance was encoded

by a similar type of plasma-mediated cephamycinase mobilized AmpC gene known as bla_{CMY2} (Winokur et al., 2000).

Plasmids are self-directed, extra chromosomal pieces of circular DNA (Lowrance et al., 2007). They hold familiar DNA that helps bacteria survive a harsh environment such as antibiotics disrupting the cell wall. Plasmids may also be transferable and harbor genes that are resistant and can be passed from one bacterium to another. However, plasmids can be the key factor deciding if bacteria are to acclimate or expire in the presence of an antibiotic drug, heavy metal, temperature, or pH change (Harvey et al., 2001). Genetic information encoded on a plasmid can assist in the survival of the cells, enabling adaptation to contrary environmental conditions such as exposure to an administered antibiotic (Kunze et al., 2005).

The increase in resistance of Gram-negative bacteria is primarily due to mobile genes on plasmids that can readily spread through bacterial populations. Standardized plasmid typing methods are enhancing our understanding of the host ranges of these elements and their worldwide distribution. Moreover, unprecedented human air travel and migration allow bacterial plasmids and clones to be transported rapidly between countries and continents. Much of this dissemination is undetected, with resistant clones carried in the normal animal and human flora and only becoming evident when they are the source of important infections. In the mid-1990s the extended-spectrum beta-lactamase (ESBL) gene encoded by $bla_{CTX\text{-}M}$ was first reported in India. The gene was transferred from the chromosome of its natural hosts, *Kluyvera* spp, to plasmids that had later spread widely, establishing $CTX\text{-}M$ as the now widely known ESBL and the primary cause of developed resistance to third-generation cephalosporins in *Enterobacteriaceae*

(Kumarasamy et al., 2010). Rates of cephalosporin resistance are lower in other countries, yet the growing prevalence of ESBL producers is sufficient to promote increased use of carbapenems. Therefore, there is selection pressure for carbapenem resistance in *Enterobacteriaceae,* and its emergence is a public health concern since there are few antibiotics in replacement for carbapenems (Kumarasamy et al., 2010).

Bacteriology

Escherichia coli (E. coli) is a difficult species to fully comprehend, having diversified into many areas of both the healthy intestinal microbiota as well as pathogenic strains, further sub-grouped into pathotypes of partly zoonotic intestinal pathogenic *E. coli* and extra intestinal pathogenic *E. coli*. The increase in antimicrobial resistant bacteria of animal origin is mostly traced back to human medicine from years ago, with variable time lags between emergence in human medicine and detection in food animals. There has been a significant increase since the 1990s, with an appearance of ESBL-producing and AmpC β-lactamase-producing *Enterobacteriaceae*, in particular *E. coli* (Ewers et al., 2012).

When discovery first occurred, ESBL and AmpC-producing bacteria were only observed in human subjects; however, recent observations of these bacteria appeared first in companion animals and they are now prevalent in livestock. This has initiated monitoring studies concentrating on food producing animals (Ewers et al., 2012). Because ESBL and AmpC-producing *E. coli* isolates are found in increasing numbers in food-producing animals, such as cattle, swine, and poultry, it is leading to concern that animals might become infection sources to harbor these bacteria and allow their emergence into the food chain, affecting public health. Resistance against β -lactams is continually being reported

and is on the rise in bacteria of the Enterobacteriaceae family isolated from both humans and animals. However, there is no specific β -lactamase associated specifically with companion or food animals because most enzymes are also predominant in bacteria found in humans (Smet et al., 2009). Modern food animal production depends upon antimicrobials for disease control and treatment. Due to the lack of understanding, food producing animals are often blamed for the increase in human pathogenic resistance to antibiotics. However, with more research, education and judicious use of antibiotics within human and veterinary medicine, these issues may better able to be understood and any associated hazards and risks thus reduced.

Pharmacology and Pharmacokinetics

The increase in prevalence of antimicrobial resistance, consumer demands and improved understanding of antimicrobial action has encouraged international agencies to review the use of antimicrobial drugs. More detailed understanding of relationships between the pharmacokinetics of antimicrobial drugs in target animal species and their action on target pathogens, has led to stricter regulations in the design of dosage labels which helps improve the activity and reduce the selection pressure for resistance in antimicrobial therapy (McKeller et al., 2004). Pharmacokinetics and pharmacodynamic (PK/PD) relationships such as modeling the area under the concentration with a time curve from 0 to 24 hours and minimum and maximum inhibitory concentration time during which plasma concentrations exceeds the lowest concentration of a chemical that prevents visible growth of at which it has bacteriostatic activity. This process has been particularly useful in optimizing efficacy and minimizing resistance (Valle et al., 2011). Antimicrobials have been categorized as: 1) concentration-dependent where increasing

concentrations at the peak of infection improve elimination of bacteria, or 2) time-dependent where exceeding the MIC for an extended percentage of the inter-dosing interval results in improved efficacy of antibiotic therapy (McKeller et al., 2004).

The emergence of resistant bacteria occurs in food animals wherever antimicrobials are used; however, whether or not agricultural antimicrobial use constitutes a substantial and quantifiable public health risk remains unknown. Opponents of agricultural antimicrobial use strongly believe that their use causes antimicrobial resistance to develop and proliferate in food animals, which results in antimicrobial-resistant organisms that contaminate the food supply and infect consumers (Jiang et al, 2006). It is believed that food animals are a target site for organisms that have genes for antimicrobial resistance; as a result, these organisms infect humans. Unfortunately, there are limited data on the frequency at which this occurs and to what extent. Ceftiofur is the only third-generation cephalosporin licensed for use in food animals in the United States and is commonly used on dairy farms to treat a variety of diseases (Tragesser et al., 2006). One reason that ceftiofur is popular among milk and beef producers is that the withdrawal time from which an animal is treated with ceftiofur to the time at which milk or meat derived from that animal can be marketed is non-existent or short, respectively, compared with other antimicrobials.

Ceftiofur Crystalline Free Acid (CCFA)

Ceftiofur is a third-generation cephalosporin which is approved for use in cattle, swine and poultry in United States. It has broad-spectrum activity against Gram-positive and Gram-negative bacteria (Kang et al., 2008). Ceftiofur's irreversible bactericidal mechanism of action is centered on a β-lactam ring in its molecular structure. All beta-

lactams have this ring. Defining feature, but not the feature that defines what ceftiofur does versus other beta lactams. This ring forms an analog of the terminal amino acid residues on precursor subunits of the peptidoglycan layer (McGowan, 2014). Due to the antibiotic's efficacy against respiratory bacteria such as *Pasteurella haemolytica*, *Pasteurella multocida* and *Haemophilus somnus*, ceftiofur sodium is approved in many countries worldwide for the treatment of bovine respiratory disease affecting beef cattle (Jiang et al., 2006). The CCFA antibiotic combines the proven efficacy of ceftiofur against bacterial respiratory pathogens with a sustained release formulation. Bacterial infections of soft tissues, such as the lung, cause changes in interstitial fluid composition, which can affect the distribution or mode of action of antibacterial in the target tissue (Hibbard et al., 2006). A study was performed to measure the drug concentrations in infected and non-infected tissue, and in plasma, lung tissue, and bronchiolar lung fluid at various times after ceftiofur administration. Previous work from Pfizer Animal Health (now known as Zoetis Animal Health) in 2004, suggested that protein-bound ceftiofur-related metabolites may serve as a reservoir for microbiologically active drug in the animal. This same protein-binding effect serves as a mechanism for transporting active metabolites into infected tissue (Johnson et al., 2004). In addition, protein binding results in increased concentrations of ceftiofur in infected compared to non-infected extracellular fluid, which is the location for the target bacterial pathogens of BRD. In one particular study, ceftiofur concentrations from plasma and infected chamber interstitial fluid were above the efficacy threshold (>0.2 µg/mL) nine days after administration of ceftiofur (Hibbard et al., 2006). A similarly designed 26-day study measured the effects of ceftiofur and chlortetracycline (CTC) treatments on antimicrobial susceptibility and

resistance genes among *E. coli* from the feces of 176 feedlot steers; CCFA treatments were either administered to all of the steers or just one individually within a pen, while chlortetracycline (CTC) was either fed or not fed to certain treatment pens (Kanwar et al., 2013). Nonspecific *E. coli* was isolated from fecal samples collected on days 0, 4, 12, and 26. The ceftiofur pen treatment was intended to mimic a metaphylactic treatment for BRD control in feeder cattle. The single animal treatment within a pen was designed to mimic treatment of clinical BRD cases among a feedlot hospital population later returned to the home pen. The ceftiofur selected isolates with reduced susceptibility towards ceftiofur on both a genotypic and phenotypic level. Results indicated that the ceftiofur treatment with either one of all of the steers resulted in a significant decrease in total resistant *E.coli* count in the feces (Kanwar et al., 2013).

Researching a mathematical model of plasmid-mediated resistance to ceftiofur in commensal enteric *E. coli* of cattle, Volkova and others (2012) tested a mathematical model of the dynamics of ceftiofur-sensitive and resistant commensal enteric *E. coli* in the absence of and during parenteral therapy with ceftiofur. This experiment indicated that a low but stable fraction of resistant enteric *E. coli* could survive in the absence of immediate ceftiofur pressure administered, continuing by horizontal and vertical transfers of plasmids carrying resistance-genes, through disrupting of *E. coli*, which was found to be resistant. During parenteral therapy with ceftiofur, resistant enteric *E. coli* increased in overall number and relative frequency. This increase was influenced by parameters of antimicrobial action of ceftiofur against *E. coli*. More than 5 weeks after the start of treatment, the fraction of ceftiofur-resistant cells among enteric *E. coli*, similar to that in the absence of treatment, was most influenced by the parameters of ecology of enteric *E.*

coli, such as the frequency of transfer of plasmids carrying resistance-genes. The results of this modeling exercise showed a 91% decrease in counts of total and of ceftiofur-sensitive enteric *E. coli*, with a concomitant increase in the fraction of resistance among *E. coli* during therapy with ceftiofur. For a 5-day therapy in a mature cow, the model showed a decrease in total *E. coli*. count from Day 1, to a minimum on Day 3, remaining decreased until Day 4, and then returning to pre-treatment concentrations on Day 7 (Volkova et al., 2012). The decrease in *E. coli* counts in the gut flora have been seen in previous research (Kanwar et al., 2013) with treatment involving ceftiofur. The impression of the mode of action with ceftiofur and the immediate drop in *E.coli* population may be due to immediate release of antibiotic from the injection site. It is meant to be a quick acting antibiotic; therefore, differences are noticed in results of fecal samples taken within hours or days of injection.

Tulathromycin

Tulathromycin has been shown to be effective against the common bacterial causes of respiratory disease in cattle and swine. Rapid uptake from the injection site promotes early achievement of MIC in lung tissue. This particular antibiotic penetrates Gram-negative bacteria, and it works by way of both bacteriostatic and bactericidal activities. It is characterized by rapid absorption from the injection site, extensive distribution to tissue, and slow elimination, allowing a prolonged drug concentration in the lungs (Evans, 2004). It could easily be hypothesized that because this is a slow elimination mode of action antibiotic, *E. coli* bacteria in the gut flora exhibited very little significance in *E. coli*/CFU counts compared to the Excede treatment.

Nutsch and others (2005) studied the clinical efficacy of tulathromycin among 720 pigs in six swine herds in North America. Feeder pigs with clinical swine respiratory disease (SRD) were randomly assigned in equal numbers to a group treated with tulathromycin given as a single dose at 2.5 mg/kg of body weight, or else to a saline-treated control group. Four of the herds studied also included a third group treated with ceftiofur sodium for 3 consecutive days at 3 mg/kg of body weight. Pigs were treated on Day 0 and evaluated for treatment response on Day 7. Within each trial, 10 or more control (nontreated) pigs and saline-treated pigs that did not respond to treatment underwent necropsies to obtain lung samples; these were then evaluated for SRD pathogens. Cure rate results were 46.4% for saline-treated pigs (control), 71.1% for tulathromycin-treated pigs, and 63.1% for ceftiofur-treated pigs. The mortality rate was significantly less for both the tulathromycin- and ceftiofur-treated pigs compared with those treated with saline (Nutsch et al., 2005).

Feedlots in Colorado, Idaho, and Texas made up the sample population of 3 trials to test the effectiveness of tulathromycin injectable solution in high-risk cattle that were highly susceptible to BRD. Each location randomly assigned 250 calves to receive tulathromycin at 2.5 mg/kg and 250 calves to receive either tilmicosin at 10 mg/kg (Colorado site) or else florfenicol at 40 mg/kg (Idaho and Texas sites) upon arrival; cattle were housed by treatment in groups of 50 head/pen. Cattle were observed daily for clinical signs of BRD until harvest. All 3 trials showed that cattle treated with tulathromycin had a significantly higher success rate at 28 days after treatment and at harvest versus other treatments of tilmicosin and florfenicol. Fewer tulathromycin treated cattle had been removed from the group at the 28-day post treatment period as

morbidities and mortalities. Tulathromycin was demonstrated to have the advantage compared with tilmicosin and florfenicol when treating groups of high-risk cattle before the onset of signs of BRD (Rooney et al., 2005).

A number of trials have been conducted evaluating the efficacy of various antibiotic treatments on BRD and other diseases. Tulathromycin, a member of the macrolide family, is a common product on the market and, therefore, producers and veterinarians are familiar with the expected response to treatment (Kilgore et al., 2005). Previous studies reported treatment with tulathromycin of naturally occurring respiratory disease in beef cattle in the United States. The relative risk of retreatment for BRD was calculated for each study included in the final review of 782 manuscripts (Kilgore et al., 2005). Results of the meta-analysis suggested that treatment with tulathromycin resulted in a 50% decrease risk of re-treatment for bovine respiratory disease compared with that of tilmicosin (Wellman and O'Connor, 2007).

Sweeny (2008) conducted a study to determine the activities of two antibacterial agents used in the treatment of bovine respiratory infections: tulathromycin, and ceftiofur. They administered the 2 antibiotics separately, in combination with each other, and in combination with each of 7 additional antibiotics. Overall, the *in vitro* data demonstrated that tulathromycin and ceftiofur, in combination with each other or seven other antimicrobial agents, primarily produce responses without synergism and rarely with antagonism. However, they did conclude that all combinations that showed synergism based on fractional inhibitory concentration (FIC) also showed synergism by time.

Pereira and others (2014) used dairy farm calf records to identify antimicrobial drug treatments in calves and to evaluate their effects on the prevalence of antimicrobial-resistant *E. coli* from rectal swabs of young weaned dairy calves. Eight farms located in the eastern United States participated in this study; three farms used individual pen housing management and five farms used group pen housing management. Three fecal *E. coli* isolates per calf were tested for susceptibility to 12 antimicrobial drugs. A total of 1,423 commensal *E. coli* isolates were tested from 473 calves. Of the 9 antimicrobial drugs used on study farms, only enrofloxacin use was significantly associated with reduced antimicrobial susceptibility of *E. coli* isolates, although previous treatment with ceftiofur was not associated with reduced *E. coli* susceptibility to ceftriaxone. Treatment with ceftiofur resulted in an odds ratio of 3.0 (95% CI: 0.9-12) for isolation of non-susceptible *E. coli* to ceftriaxone compared with calves not treated with ceftiofur. Treatment with enrofloxacin resulted in selection of isolates that presented phenotypic resistance to both ciprofloxacin and ceftriaxone. Treatment with ceftiofur resulted in a higher prevalence of isolates resistant to ≥3 antimicrobial drugs (97%) compared with no treatment with ceftiofur (73%). These findings reinforce the necessity for continued implementation of practices at the dairy farm that support the sustainable and judicious use of antimicrobial drugs in dairy calves (Pereira et al., 2014).

Resistance due to Extended Spectrum Beta-Lactamases

Extended spectrum beta-lactamases (ESBL) are enzymes of Gram-negative bacteria conferring resistance against β-lactam antibiotics, such as third- or fourth-generation cephalosporins and monobactams. ESBL-producing Gram-negative bacteria have been isolated worldwide.

Most ESBL-producing bacteria are multidrug-resistant, and the majority of them are susceptible only to carbapenems and colistin. Infections caused by these multidrug-resistant bacteria therefore are associated with high morbidity and mortality, as well as high health care costs and limited therapeutic options. Resistance genes of the ESBL type are mostly plasmid-mediated, and therefore can transfer among bacteria. Recently, there has been an ongoing concern about the dissemination of ESBL-producing strains in healthy food animals, with many reports referring to strains from Europe, Asia, and the United States. The increasing issue of infection or colonization with ESBL-producing *E. coli* has been observed in food animals such as cattle, broiler chickens, and pigs. This observation suggests that animals, food, and environment are potential sources of ESBL-producing bacteria (Economou and Gousia, 2015).

Beta-lactamases

Beta-lactamases are enzymes formed by bacteria that provide multi-resistance to beta-lactam antibiotics such as penicillins, cephamycins, and carbapenems, although carbapenems are generally resistant to most beta-lactamases (Jarlier et al., 1988). Beta-lactamases are enzymes that cleave the beta-lactam ring, thereafter deactivating the antibiotic. The first plasmid-mediated beta-lactamase in Gram-negative bacteria was discovered in Greece in the 1960s. It was named TEM after the patient from whom it was isolated, “Temoniera” (Berkowitz and Mechock, 1995). Subsequently, a closely related enzyme was discovered and named TEM-2, and so on thereafter. TEM-2 was identical in biochemical properties to the more common TEM-1, differing only by a single amino acid with a resulting change in the isoelectric point of the enzyme (Paterson and Bonomo, 2005). β-Lactam antibiotics are the most commonly prescribed antibiotics in the world.

Therefore, it is not surprising that resistance to this very important class of antibiotic poses an increasingly complex issue for physicians. ESBLs are a wide spread mechanism of resistance in Gram-negative bacteria and is by far the single most important factor pertaining to antibiotic usage and resistance in the body (Paterson and Bonomo, 2005). With the introduction of original first and second generation β-lactam elements, noticeable changes in β-lactamases include the increased dominance of older enzymes and also the appearance of new enzymes, and differing levels in the expression of enzymes. These changes have been responsible for resistance to new and improved cephalosporins, monobactams, carbapenems, and β-lactamase inhibitor (e.g., clavulanic acid) and β-lactam drug combinations.

Beta-lactamases Mechanisms and Mode of Action

Beta-lactam's mode of action is defined by inhibiting the bacterial cell wall formation, thereby disrupting the cell wall of the microorganism. ß-lactams are a class of broad-spectrum antibiotics, including of all antibiotic agents that contain a beta-lactam ring in their molecular structures; this includes: 1) penicillin derivatives, such as penicillin G, penicillin V, methicillin, oxacillin, nafcillin, ampicillin, amoxicillin and carbenicillin, 2) cephalosporins, such as cephalothin, cefamandole, ceftiofur, cefotaxime, and cefepime, 3) monobactams, such as aztreonam, and 4) carbapenems such as imipenem, ertapenem, and meropenem). Generally, there is more emphasis on the penicillins and cephalosporins (Holton et al., 2000). In 2003, over half of the commercially available antibiotics in use in human medicine were β-lactam compounds (Elander, 2003). β-lactam antibiotics are indicated for the prevention and treatment of bacterial infections caused by susceptible organisms. At first, β-lactam antibiotics only

recognized to actively fight against Gram-positive bacteria; however, the recent development of broad-spectrum β-lactam antibiotics active against various Gram-negative organisms has increased their effectiveness and usefulness. To overcome emerging resistance, β-lactam antibiotics are often given with β-lactamase inhibitors such as clavulanic acid. However, this only works for certain beta-lactamases (e.g., ESBLs but not AmpC).

Resistance to β-lactam antibiotics can also develop via modifications in the cellular targets of the antibiotics, such as the penicillin-binding proteins, and through changse in the outer membrane permeability of the organisms to the antibiotics (Berkowitz and Mechock, 1995). Defining resistance due to ESBLs in the laboratory is one of the clinical challenges with ESBL-producing organisms. These organisms may appear susceptible to specific antimicrobial agents at a lower colony forming unit concentrations (CFU). However, they may have significantly increased MIC to the same agents at higher inoculums of 10^7 or 10^8 (Patterson, 2002). This inoculum effect is noted with the third-generation cephalosporins: cefotaxime, ceftriaxone, and ceftazidime, as well as the fourth-generation cephalosporin, cefepime. These drugs bind to and inhibit the carboxypeptidases and transpeptidases (Patterson, 2002). By their mode of action, the antibiotics attacked and kill susceptible bacteria allowing only resistant bacteria to survive (Jarlier et al., 1988). Antimicrobials are known to be harmful vectors that inhibit homeostasis in the bacterial cellular environment. The bacteria cell that is not resistant to antibiotics will quickly surrender to the pressure of the drugs (Kunze et al., 2005). On the other hand, resistance may quickly be conferred through mutation or DNA transfer, often stimulated further by the presence of antibiotics, thereby quickly recruiting new strains of

bacteria into the resistant sub-population. There are many ways resistant bacteria may obtain these traits to function as resistant, which include plasmids, pre-programmed genetic information, and surviving unfavorable environments that aids in immunity for the actual bacteria (Kunze et al., 2005). Functionally, β-lactam resistance may be a result of the production of β-lactamases, impermeability, efflux and target modification. These modalities may occur singly or in different combinations (Schentag, 2002).

An understanding of β-lactamase detection and identification is therefore invaluable. Accurate identification of the β-lactamases of Enterobacteriaceae often requires gene or protein sequencing; however, often the broad category of enzyme produced by an isolate can be inferred from phenotypic antibiotic susceptibility patterns (Choi et al., 2008). Resistance to ceftazidime or cefpodoxime implies that the ESBL production in *E.coli* and *Klebsiella* spp., especially if susceptibility to cefoxitin is retained (whose resistance points to an AmpC producing bacterium). ESBL production can be confirmed with double disc tests (e.g., synergy of the drug with clavulanic acid restores susceptibility) or with various commercial kits.

Numerous risk factors for infection have been reported from case-control studies of various outbreaks of ESBL-producing organisms. Reports of risk factors include: the presence of an intravascular catheter, gastrostomy tube, emergency intra-abdominal surgery, gastrointestinal colonization, length of hospital or ICU stay, prior antibiotic use (including third-generation cephalosporins), severity of illness, the presence of a urinary catheter, ventilator assistance, and long term assisted living (Patterson, 2002). Because different outcomes are present with each type of risk, different methods for prevention and treatment should be discovered. Research has shown that greater exposure to broad-

spectrum cephalosporins and exposure to trimethoprim and sulfamethoxazole and aminoglycosides were significant risk factors for ESBL-producing *K. pneumoniae* or *E. coli*, and total exposure to antimicrobial agents was the only independent analysis of infection with an ESBL-producing organism.

The treatment of infections caused by antibiotic-resistant bacteria is one of the great challenges faced by medical doctors in the 21st century. A study took place to understand the ability of the plasmid *pCT*, a globally distributed plasmid that harbors an ESBL resistance gene ($bla_{\text{CTX-M-14}}$), to continue to spread in the absence of antibiotic pressure. Key characteristics of plasmid success include conjugation frequencies, bacterial-host growth rates, ability to cause infection, and impact on the efficiency of host strains (Cottell et al., 2013). It is understood that the prevalence of $bla_{\text{CTX-M}}$ is expanding among *E. coli* strains in feedlots across the United States. It is rapidly transferred through pathways among via plasmids and through vertical transmission (clonal transmission) among comingled animals (Cottell et al., 2013).

Although the pathway of ESBL into US cattle feedlots has been detected, there is a lingering knowledge gap that inhibits scientists' abilities to fully understand the complexity of the spread of extended spectrum ß-lactamases, how they are effective and the reasons why they correspond with antibiotic resistance relative to *E. coli*. Because of the complex nature of outbreaks due to ESBL-producing organisms as already reviewed, additional control measures including antibiotic utilization should be considered for critically important antibiotics such as third and fourth generation cephalosporins, extra-label drug usage, as well as public control methods in public health care systems, in addition to overall proper food handling and safety. Although there are suggestions about

completely eliminating the use of cephalosporins in animal agriculture (Liebana et al., 2013), and the statistical effectiveness of individually reducing public health risks, there are no data to prove this approach would work. Antibiotic utilization patterns, including widespread cephalosporin use and particularly the extensive use of ceftazidime in human medicine, have been associated with the emergence of ESBLs in public health (Patterson, 2002). It is not at all implausible that a similar spread in food animals has been facilitated by the use of ceftiofur.

There is a clear need for research input from a range of disciplines, not only clinical and veterinary medicine, epidemiology, microbiology and pharmacology, but also health economics, international law and social science. Action on resistance will require cooperation from governments, industry and international agencies as well as scientists. That action will need to involve all whom it will affect such as, clinicians, pharmacists, patients, veterinarians and farmers; all of whom have contributed to the current antimicrobial resistance problem and each of whom will need to be part of a long-term solution. The most important need is to develop strategies for improved antimicrobial stewardship in both human medicine and agriculture, and to create an effective alternative approach to reduce infectious disease in both humans and livestock.

Objectives of Research

We aimed to determine the effects of therapeutic regimens of ceftiofur crystalline free acid and tulathromycin on resistance in *E.coli* bacteria; specifically, to extended-spectrum beta-lactamases (ESBL) and mobilized AmpC beta-lactamases in feedlot cattle. To do so, we measured the relative and absolute sample-level and bacterial level prevalence of phenotypic and genotypic resistance to third-generation cephalosporins in

beef feedlot cattle before, during and after treatment with antibiotics, and including up to the stage of slaughter.

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CHAPTER II

Prevalence of Extended-Spectrum Beta Lactamase Producing Coliform Bacteria in Fed Cattle Receiving Metaphylaxis

Introduction

Antimicrobial resistance is the capability of a specific bacterium to resist the effects of an antimicrobial (or, most often, an antibiotic) used to prevent and treat bacterial infections. Resistance develops through either innate resistance, genetic mutation, or from a bacterium acquiring resistance transferred from another. Because of random mutations, resistance emerges occur sporadically. Its success in expanding and spreading depends on selective pressure. This can occur from gradual build up in resistance due to overuse or misuse, or simply any use, of the actual antibiotic or antimicrobials. Antibiotics used in food-producing animals are used for 4 primary reasons: treatment, control, and prevention of bacterial infectious diseases (so-called therapeutic indications) and for growth promotion and feed efficiency (Schwarz and Chaslus-Dancla, 2001). The relatively short period of time between the introduction of an antimicrobial agent into use and the first observations of resistant bacteria confirms that bacteria are able to quickly and efficiently acclimate to the changing environmental conditions in the host. Bacteria have also developed highly efficient ways to transfer resistance genes between members of different families, genera, and species of bacteria. These transfer mechanisms allow a rapid exchange of resistance genes within mixed

bacterial populations commonly seen on the skin but also on the mucosal surfaces of the respiratory, alimentary and genito-urinary tract of humans and animals.

Escherichia coli (*E. coli*) is a member of the family Enterobacteriaceae. Almost all members of this family of bacteria are part of the normal intestinal flora of animals and humans, though there are many strains that are pathogenic to animals and humans, and certain *E. coli* are an emerging cause of foodborne illnesses. *E. coli* is a Gram-negative, oxidase-negative, non-spore forming, non-acid fast rod. It also thrives in both aerobic and anaerobic environments and is tolerant to harsh environmental conditions.

Extended Spectrum β-Lactamases, ESBLs, are enzymes that confer resistance to penicillins, extended-spectrum third and fourth generation cephalosporins, and monobactams. ESBL harboring Enterobacteriaceae have emerged as some of the most significant life-threatening infections in human medicine, with *E. coli* and *Klebsiella* spp. being the main pathogens. Occurrence of ESBL-producing isolates has important clinical and therapeutic implications since resistance determinants for ESBL production are carried on plasmids that can be easily spread from organism to organism, and the spread of resistance against extended-spectrum cephalosporins may lead to increased prescription of more broad-spectrum and expensive drugs to cure the infections. Early detection of the spread of these bacteria is going to be key in order to eliminate future severe cases in both human and veterinary medicine.

The study objective herein was to determine the prevalence of ESBL and AmpC fecal *E. coli* populations in feedlot cattle administered ceftiofur, tulathromycin or control treatments via metaphylaxis. We further aimed to understand the antimicrobial resistance

patterns of *E. coli* populations at periodic times during finishing and at slaughter, and to compare prevalence and antimicrobial resistance of *E. coli* in the fecal matter among treatment groups and over time.

Materials and Methods

A total of one hundred thirty-four crossbred beef steers (initial BW = 390 ± 18.4 kg) were used in a randomized complete block design. Steers were acquired from 2 sources located near Abilene, TX (n=99; 429.7 km), and Hereford, TX (n=35; 46.7 km) and were transported to the West Texas A&M University Research Feedlot in Canyon, TX. All cattle were previously backgrounded and arrived in good health condition. Upon arrival of both sources, (Day -3) cattle were visually identified with an ear tag in the left ear along with matching electronic identification tag in the right ear; also, each was administered a growth implant (Revalor 200, Merck Animal Health, Kenilworth, N.J.) in the caudal aspect of the right ear, an anthelmintic was administered at 200 mg/kg of BW (Dectomax, Zoetis), and BW was recorded.

Concluding group processing, cattle were blocked by source and BW and assigned to treatment pens (11 to 12 head per pen) with 1 or2 steers randomly selected to be a sentinel control and received no antibiotic to test for resistance among treatment within the pen. Each of the 3 treatments were replicated 4 times. The trial began on d 0 with fecal samples collected in a plastic sleeve, labeled and immediately chilled. Once all samples were taken, the sleeves were packed in shipping coolers with ice packs and shipped to the lab in College Station, TX where further analysis occurred. Immediately after their initial fecal sample was collected, the cattle received 1 of 3 treatments (per pen

and within-pen randomization scheme) consisting of: 1) control, no antimicrobial administered; 2) ceftiofur (Excede, Zoetis, Kalamazoo, MI) administered 6.6 mg/kg BW in caudal aspect of the left ear; and 3) tulathromycin (Draxxin, Zoetis) administered 2.5 mg/kg BW subcutaneously in the neck. A blank colored ear tag (consisting of yellow, pink, light green, blue, silver, purple, orange, red, and teal) was assigned and placed in the right ear of each animal that belonged to each assigned pen to insure all steers were replaced in the correct pen assignments daily. Cattle were then provided a starter ration containing a mineral supplement without antibiotic, and later receiving the final finishing ration with a mineral supplement that also did not include antibiotic (Table 2A and 2B).

Sample collections

Approximately 25 g of fecal matter was collected from the rectum via obstetric sleeve glove and turned inside out and labeled with the 4-digit identification number that matched the animal ID. Samples were collected and BW were recorded on days 7, 14, 28, 56, 99, and the day before block slaughter date. Hide swab samples (1 m^2 area) were collected pre-shipping for each slaughter sampling period, and carcass data and bilateral subiliac lymph nodes were collected at slaughter from all steers. Feces, hide swabs, and lymph nodes were chilled and shipped on ice at 4°C and further processing took place at Texas A&M University (College Station, TX).

Fecal samples from each sampling period were mixed at 1:1 ratio of glycerol and 5 mL of the mixture was placed in cryo-vials and stored at -80ºC for subsequent bacteria isolation and evaluation for presence of *E. coli*. This process took place through enrichment and plating to selective media. *E. coli* positive fecal samples were identified

by enrichment through plating onto MacConkey (MAC), MacConkey plus ceftriaxone (4 µg/ml) (MAC-CEF) agar, and following an overnight broth enrichment at 2 ug/ml ceftriaxone in MacConkey Broth, later plated to ESBL-Chrome agar.

Quantification of the bacteria analysis procedure was carried out in the lab on a spiral-plater. Initial process began with a 0.5 g of fecal matter weighed from frozen cryo-vial sampling tubes with a 1:1 ratio of glycerol tubes and suspended in 4.5 mL of sterile PBS solution, then homogenized by vortexing. From each suspension, 50 µl was spiral plated onto plain MacConkey plates (Mac) and Mac with 4 mg/L ceftriaxone (MAC-CEF), respectively, and were then incubated at 37ºC for 18 to 24 hours. Colonies that grew on MAC and MACEF were colony counted using the automated (Flash n Go Colony Counter, Neutec, Farmingdale, NY) and streaked on blood agar plates for indole testing. One colony was selected from Mac plates and two colonies were selected from MAC-CEF plates. 50 µl of MACEF broth enrichment was spiral plated on Chrome agar (ESBL) plates and incubated for 18 to 24 h at 37 ºC. Indole tests were performed on isolates from MAC and MACEF plates to confirm as *E. coli* and indole positives were preserved into cryo-beads.

In addition, 0.5 mL of the original suspension was transferred into 4.5 mL of MacConkey broth supplemented with ceftriaxone (2 mg/L) and these tubes were incubated at 37 ºC for 18- to 24 hours. Following this additional enrichment step, 50 µl was spiral-plated to CHROM-ESBL (Hardy Diagnostics and incubated as above. After the incubation of ESBL plates; dark pink to reddish colonies identified from CHROM-ESBL plates were considered as *E. coli* whereas the blue colonies were considered as *Klebsiella.* The *E. coli* colonies were streaked on blood agar. Five colonies were selected

from each Chrome ESBL plate if sufficient colony growth allowed. Indole testing was performed on the isolates phenotypically characteristic of *E. coli*. Isolates from ESBL plates and the positive isolates were preserved into cryo-beads. Off color recorded isolates were identified and went through the process again for a closer examination of potential growth. This process was repeated until all samples were completed.

Phenotypical *E. coli* colonies that grew on MAC, MAC-CEF and CHROM-ESBL agar plates were picked and streaked onto blood agar plates for indole testing by using James® reagent. Indole positive isolates were placed into the cryo-beads and preserved in -80 ºC for further analyses. Later, isolates obtained from CHROMagar ESBL plates were streaked onto blood agar and these isolates were identified as *E. coli* by using Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (Bruker, MALDI-TOF MS) in species level. *E. coli* isolates confirmed my MALDI-TOF MS were tested for phenotypic antimicrobial susceptibility. Minimum inhibitory concentrations (MIC) for *E. coli* were determined by broth microdilution method using TREK Diagnostic Systems™ and Gram-negative NARMS plate (CMV3AGNF) according to the manufacturer's directions. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) breakpoints by using a plate reader (Trek Sensititre systems™). Isolates resistant more than or equal to 3 antibiotic classes were classified as multi-drug resistant (MDR). Isolates that were resistant to 3rd generation cephalosporins (i.e., ceftriaxone and ceftiofur) were presumed to be either ESBL or AmpC carriers. Among these, if the isolates was resistant to cefoxitin (2nd generation cephalosporin) it was assumed to be a cephamycinase such as $bla_{\text{CMY-2}}$. If it was susceptible to cefoxitin

and to amoxicillin/clavulanic acid, it was classified as a presumptive ESBL isolates (e.g., *bla*$_{\text{CTX-M}}$).

Statistical analysis

Performance and carcass data were analyzed as a randomized complete block design using the PROC MIXED procedure in (SAS 9.4 Inst., Inc., Cary, NC). Pen was considered the experimental unit. The class statement included pen, block, treatment, and identification number. Body weight and average daily gain were included in the model statement with a random effect of block x treatment. An alpha level of 0.05 was used; whereas, tendencies were considered for a *P*-value between 0.06 and 0.10 (Table 2.2; Table 2.3).

Stata version 15.0 was used to analyze the phenotypic resistance data. Prevalence of presumptive ESBL/AmpC isolates was calculated, including 95% confidence intervals overall, by sampling day, by treatment group, and the interaction of treatment by day. Mixed logistic regression models were used to assess full-factorial designs, with subsequent comparisons by period and treatment where interaction terms were non-significant (P>0.05). In addition, mixed logistic regession was used to also assess the probability of MDR strains (among ESBL/AmpC) as above. Finally, utilizing the difference in Log10 CFU differences between growth (as measured by spiral plating) on MAC versus MAC-CEF, and by taking the antilog for illustrative purposes, we employed a similar mixed linear modeling framework to assess the quantity of presumptive ESBL/AmpC bacteria in the same samples, though not their specific phenotype.

Results and Discussion

There was no difference ($P = 0.43$) in BW or health variables among treatment groups. Futhermore, no differences ($P = 0.43$) were observed for any of the carcass data. There was a tendency ($P = 0.10$) for ADG to differ from d 14 to 28. The Excede® group gained 1.60 kg/d compared to 1.96 and 2.12 kg/d for Control and Draxxin®, respectively. This slight difference in performance could be associated with difference the disturbance of bacteria populations in relation to administration of antibiotic. Different results may have been observed if cattle presented a health challenge or mixed sources. The efficacy of metaphylaxis is proven with research through several studies and reports. In 2010, (Watts et al.) described how antimicrobial agents have vastly improved the efficiency and performance of at-risk cattle since 1988. Another study completed in Australia showed that metaphylaxis was successful in reducing BRD and improved overall morbidity. The study included feedlot entry of tilmicosin, long acting oxytetracycline or no antibiotic treatment. An analysis of growth rate, disease occurrence and mortality were made between the groups from time of arrival and time of harvest. Conclusions indicated cattle medicated with tilmicosin at 10 mg/kg body weight on arrival, 0.08 kg/d faster than cattle medicated with oxytetracycline at 20 mg/kg body weight and non-medicated cattle. No significant differences in growth rate between medicated and non-medicated oxytetracycline cattle. However, cattle that were administered tilmicosin on arrival had 8 fewer cases of disease per 100 animals compared with cattle not treated (Cusack, 2004).

Approximately 804 feces samples were collected and evaluated for *E. coli* bacteria, which were then further evaluated for AmpC/ESBLs. Extended spectrum ß-lactamase and AmpC bacteria were prevalent in the fecal samples across all days and

treatment groups. There was no difference ($P > 0.05$) between treatment groups across days in terms of sample-level prevalence of ESBL /AmpC *E. coli*. A significant difference ($P < 0.05$) was observed, however, in the bacterial level prevalence and quantity of bacteria growing on MAC-CEF plates only on Day 7 for the ceftiofur treated group, where total coliform counts were significantly reduced (Figure 2.1). On Day 14 we reported that coliform counts had resumed to their normal level such as they were before antibiotic administration (Figure 2.2). At time of harvest, all treatment groups showed no differences among treatments ($P > 0.05$). Among the samples, 143 isolates within the ESBL/AmpC bacteria showed resistance to up to 9 classes of antibiotics and in a single isolates up to 13 (out of 14) individual antibiotics (Figure 2.3). Of these isolates, based on phenotypic resistance patterns, there appeared to 42, ESBLs and 101 producing *E. coli* (Figure 2.4).

This study was designed to explore and better understand the antimicrobial resistance treatment effects of metaphylaxis on *E. coli*, and more specifically, extended spectrum ß-Lactamases and AmpC producing bacteria in feces. Treatments in this study included ceftiofur crystalline-free acid (Excede®), tulathromycin (Draxxin®), and no treatment (Control). All treatments on Day 0 began at a similar level of total population *E. coli* counts, both overall and cephalosporin resisatnt. Similar studies have evaluated the effect of ceftiofur on cephalosporin resistance among enteric bacteria in cattle (Kanwar et al., 2013). The results from this particular trial, showing that ceftiofur has a significant effect on *E. coli* in cattle during days immediately following treatment; this parallels results from previous studies. A trial (Schmidt et al., 2013) showed similar data with the ceftiofur resistant *E. coli* immediately following ceftiofur administration. They

report that herd *E. coli* fecal and hide prevalence throughout the residency of cattle at a feedlot, including during the period of greatest ceftiofur use at the feedlot, were either not significantly different or significantly less than the initial prevalence upon arrival. Longitudinal sampling of cattle treated with ceftiofur demonstrated that once the transient increase of *E. coli* shedding that follows ceftiofur injection decreased, ceftiofur-injected cattle were no more likely than untreated members of the same herd to shed *E. coli* (Schmidt et al., 2013). Another study showed the frequency distribution of the *E. coli* isolates by the phenotypic multi-drug resistance count for all treatments (ceftiofur and chlortetracycline). The experimental unit that received ceftiofur, presented data that showed a stronger antimicrobial selection pressure and strong effects on the enteric bacteria in cattle. Ceftiofur treatment on day 0 selected for isolates with reduced susceptibility towards matching antibiotic resistance of ceftiofur. It tested for both a phenotypic and genotypic level (Kanwar et al., 2013).

Lowrance and others (2007) attempted to determine the extent and duration on numbers and antimicrobial drug susceptibility of *E. coli* in feedlot cattle. Susceptibility to 15 antimicrobials were determined with resistance to approximately 68.4% of antibiotics. The ceftiofur administration resulted in a temporary decline in ceftiofur resistant *E. coli*, yet returned to normal levels (before administration of antibiotic) within 14 days of a single dose. Comparable results with the data we have collected among the feedlot steers, and noticeable decrease in *E. coli* counts on Day 7; and then a gradual increase to pre-treatment level was recorded.

The selection pressure conferred by the use of antibiotics is generally considered to result in the amplification, dissemination, propagation and persistance of resistant

strains. The *E. coli* bacterial counts dropped significantly in the treated animals, reflecting an immediate and transient disappearance of the antibiotic-susceptible strains. The reduction implies that the susceptible population would have outnumbered the resistant population by 4 to 5 logs prior to treatment.

The results of this study and the previous studies mentioned, in which ceftiofur-resistant *E. coli* appeared expanded for a short duration, following the use of ceftiofur in cattle, and that overall *E. coli* populations declined immediately after treatment. In one investigation of dairy farms, those dairies that used ceftiofur were significantly more likely to have cows shedding *E. coli* with reduced susceptibility to cephalosporins (Tragesser et al., 2006). However, that effect was only for use/no use at the farm level; importantly, they saw no significant differences among the levels of use in those farms that employed ceftiofur.

The importance and value of antibiotics cannot be overlooked. We are a part of a world today that is totally dependent on them for the treatment of infectious diseases. In addition to their use in the treatment of infectious diseases, antibiotics are critical to the success of advances of human medicine such as for hip replacements, abdominal surgery, and many other things we take for granted. With expanding guidelines to control antibiotic usage, because of documented reductions in the availability of effective medicines, there is no question that antibiotic resistance is becoming a global issue. Just as there is no question as to resistance mechanisms and their mode of action, these create an enormous clinical and financial burden on health care systems worldwide. Not all bacterial pathogens are resistant to every antibiotic that can be administered, and many respond to trial experiments with antimicrobial agents used in the human medicine. It is

important for both human medicine and veterinary medicine to understand that we should always be pushing forward to find new and improved antimicrobial agents. Presently, knowledge of inhibitor-target and inhibitor-resistance interactions is not at the point where effective new compounds can be designed or screened with confidence; more studies of these processes at the structural level will surely provide new leads. Systems biology approaches are uncovering new types of metabolic interactions and providing non-reductionist explanations for many aspects of antibiotic modes of action and resistance (Davies and Davies, 2010).

Obviously, there is no perfect antibiotic; that is, one to cure all infectious diseases. It is going to take years and years of research and development to fully understand resistance and help the cause by getting all heath care providers, both human and animal, to have the same goal as to reducing infections, eliminating diseases all while reducing resistance. The knowledge of environmental reservoirs of resistance is becoming more apparent and known to health care researchers. With the technology and understanding that is available it is possible to detect and make aware of early developed, or potential resistance mechanisms to new or old antibiotics and thus prepare for problems in the clinic in a timely manner. With the evidence of data it is mandatory on providers, researchers, and admins of medicine to renew a concerted effort that takes full advantage of new understanding and technologies, and to use previous research to understand and reduce antibiotic resistance.

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Table 2.1A. Composition of the starter diet fed to feedlot steers.

Ingredient, % DM	Amount
Sweet Bran –Wet corn gluten feed	58.817
Corn Stover –Chopped stalks	19.199
Corn grain – Steamed flaked corn	15.285
Nutrabase 12	3.049
Supplement[1]	3.65
Calculated nutrient composition[1]	
DM, %	66.243
TDN, %	23.05
CP, %	16.367
NDF, %	31.672
Ca, %	1.138
P, %	0.717
S, %	0.334
NEm, Mcal/kg	0.406
NEg, Mcal/kg	0.271

Table 2.1B. Composition of the finishing diet fed to feedlot steers.

Ingredient, % DM	Amount
Sweet Bran –Wet corn gluten feed	49.35
Corn Stover –Chopped stalks	12.00
Corn grain – Steamed flaked corn	30.00
Nutrabase 12	3.00
Supplement[1]	3.65
Calculated nutrient composition[1]	
DM, %	68.919
TDN, %	35.94
CP, %	15.261
NDF, %	10.44
Ca, %	1.102
P, %	0.603
S, %	0.063
NEm, Mcal/kg	0.465
NEg, Mcal/kg	0.323

Table 2.2. Effect of treatment on performance of feedlot steers

Item	Treatment[1]				
	Control	Draxxin	Excede	SEM	*P*-value
BW, kg					
Initial[2]	393.6	388.9	389.8	16.8	0.44
Day 7	412.6	407.1	415.0	17.4	0.43
Day 14	439.2	433.0	439.7	19.3	0.43
Day 28	466.6	462.8	462.2	20.7	0.44
Day 56	525.0	522.5	524.2	20.1	0.45
Day 99	584.2	580.9	584.9	20.9	0.45
Final BW	635.7	631.2	635.5	5.94	0.38
ADG, kg/d					
D 0 to 14	3.24	3.14	3.56	0.24	0.21
D 0 to 28	2.60	2.63	2.58	0.16	0.44
D 0 to 56	2.36	2.37	2.40	0.08	0.41
D 0 to 99	1.92	1.93	1.97	0.06	0.37
D 14 to 28	1.96	2.12	1.60	0.20	0.10
D 28 to 56	2.13	2.08	2.22	0.13	0.33
D 56 to 99	1.34	1.35	1.40	0.07	0.35

Table 2.3. Effect of treatment on carcass traits of feedlot steers

Item	Treatment[1]				*P*-value
	Control	Draxxin	Excede	SEM	
HCW, kg	390.3	391.3	392.8	13.4	0.43
CALCYG	3.24	3.29	3.28	0.14	0.96
REA	13.0	13.0	13.0	0.40	0.99
Marbling Score	397.9	384.3	409.7	18.6	0.63
BFA	0.50	0.49	0.50	0.02	0.95
KPH, %	1.83	1.85	1.84	0.72	0.97

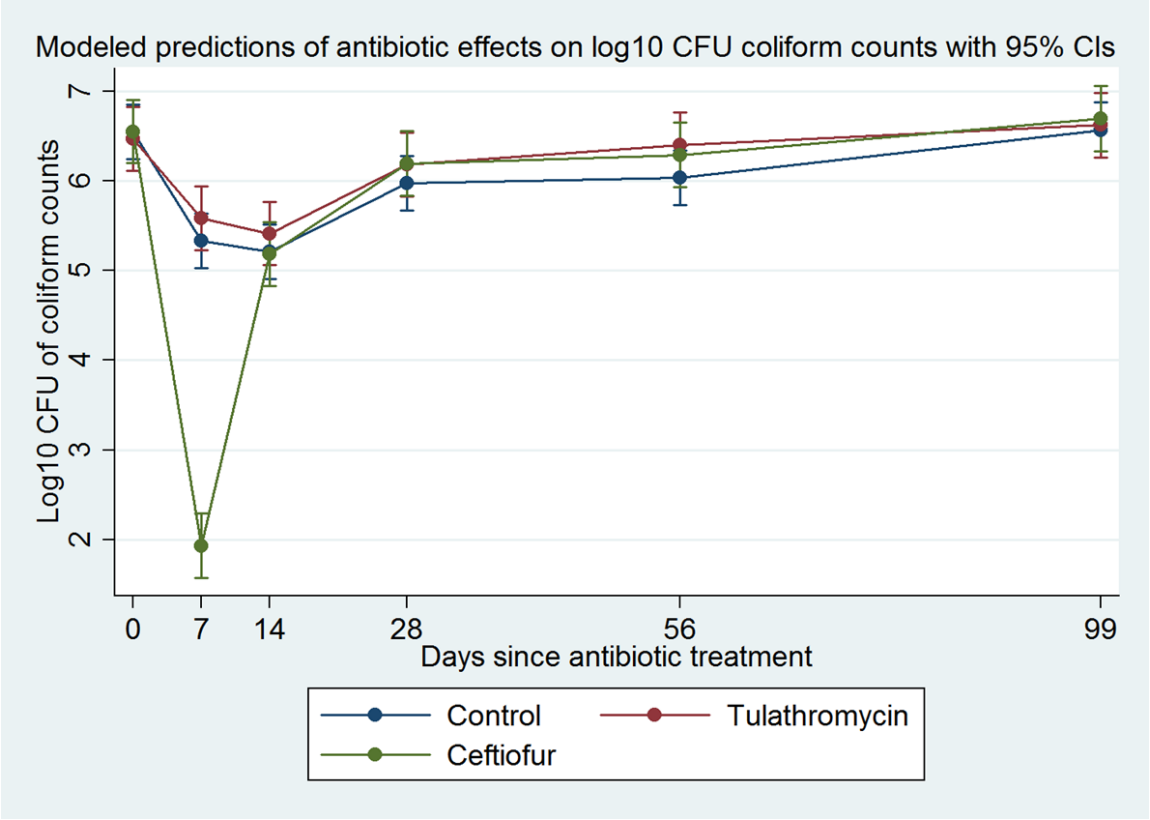

Figure 2.1. Cephem-r bacterial model predictions. Proportion of total E. coli population that exhibits resistance to 3rd generation cephalosporins.

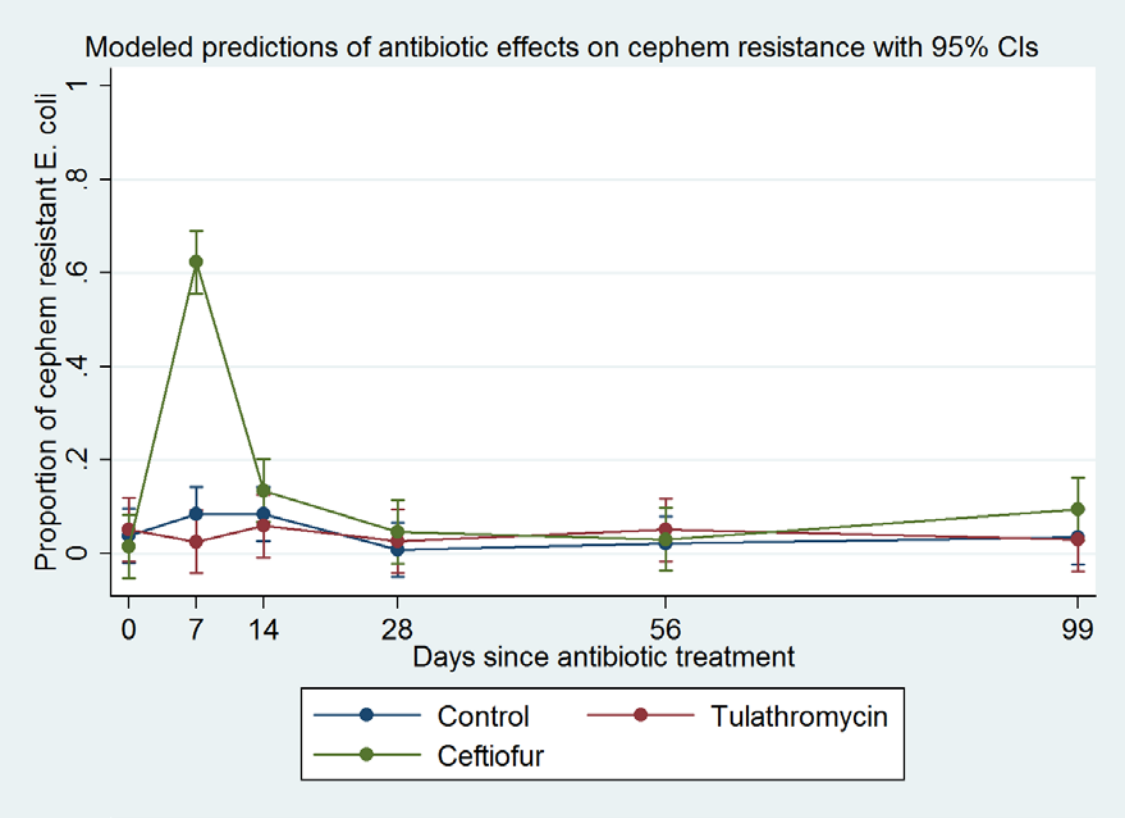

Figure 2.2. Modeled predictions of antibiotic effects of treatment by day. In this graphic, the effects of treatments on total CFU of all E. coli in a gram of feces is modeled

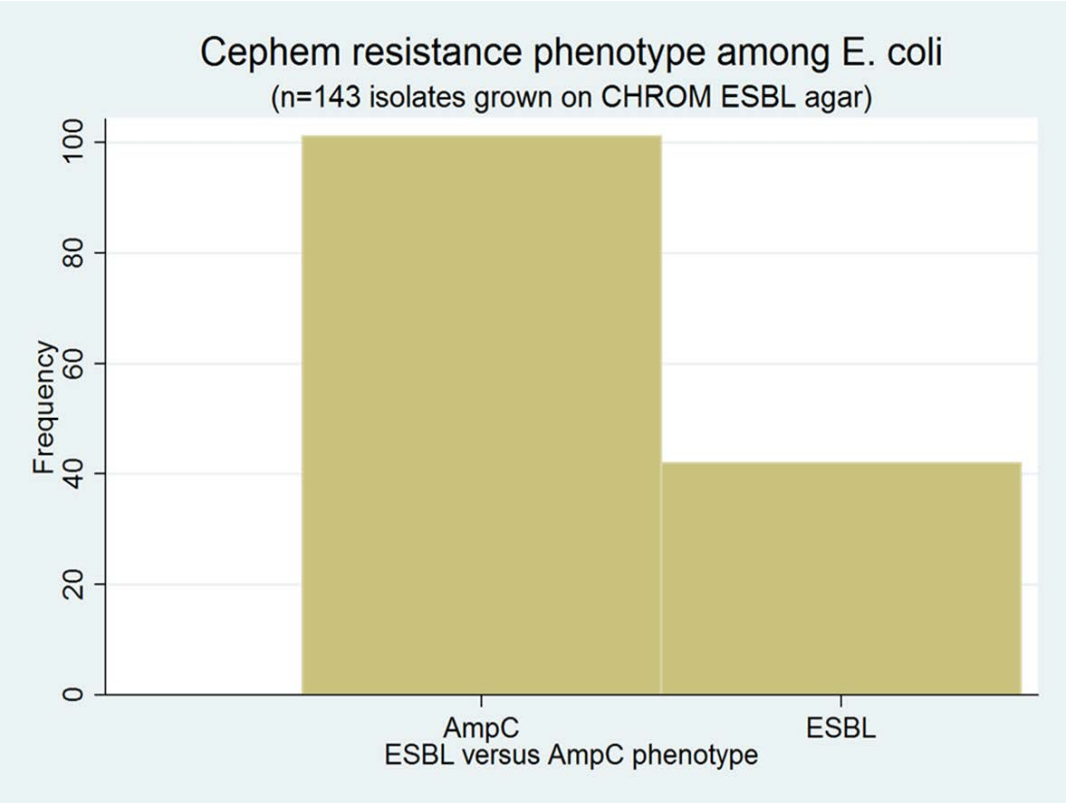

Figure 2.3. ESBL and AmpC cephem resistance among E. coli. Forty-one isolates were of the ESBL phenotype while 101 were of AmpC.

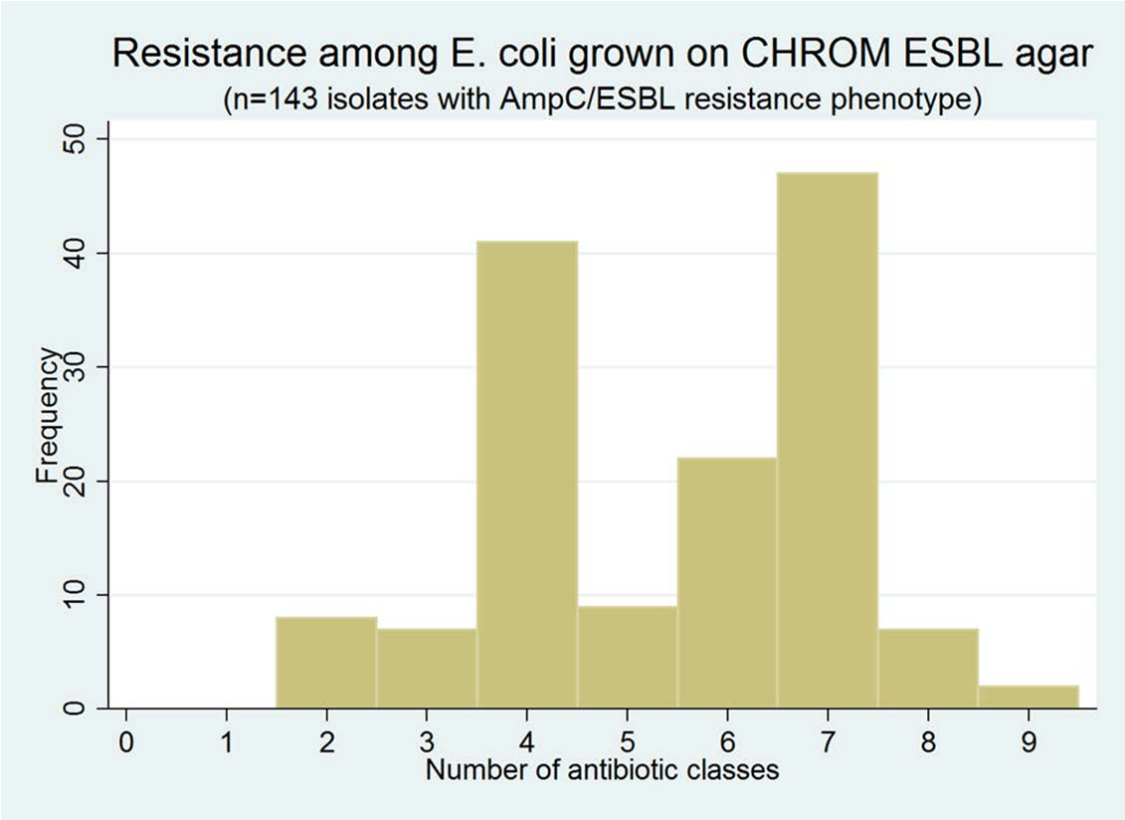

Figure 2.4. Multi-drug resistance among *E. coli* grown on CHROM ESBL agar. Bars represent the count of antibiotics to which each of the cephalosporin isolates (n=143) was resistant.

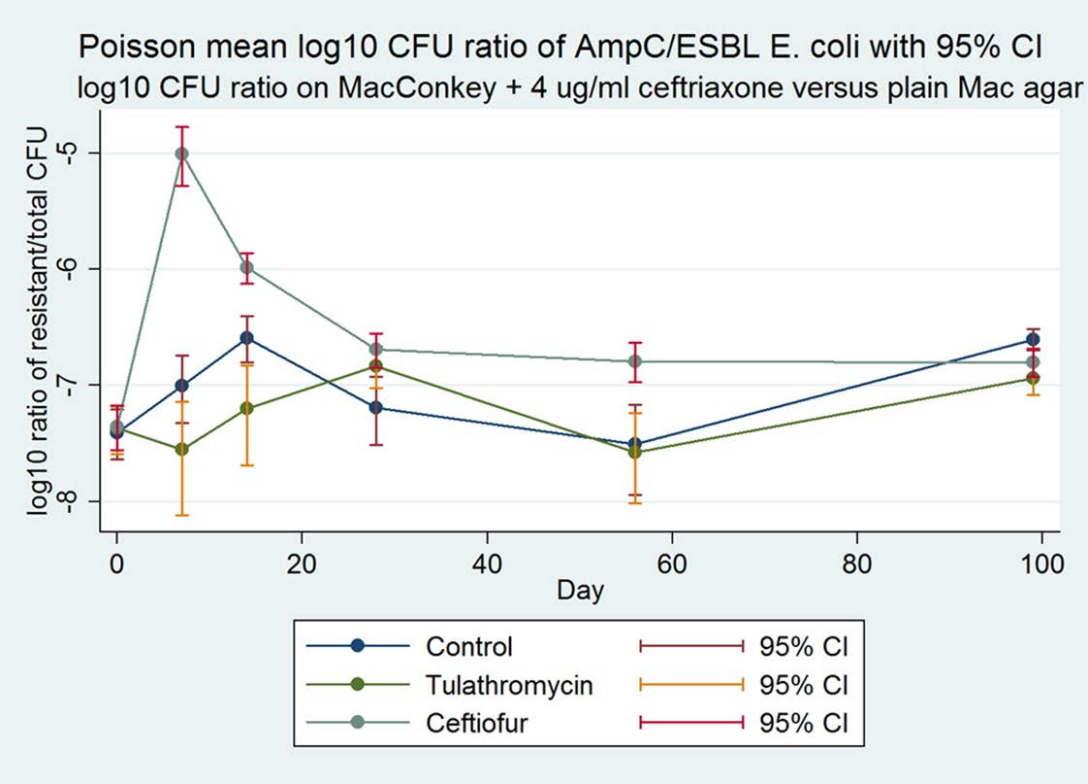

Figure 2.5. Poisson mean log 10 ratio of AmpC and ESBL *E. coli*

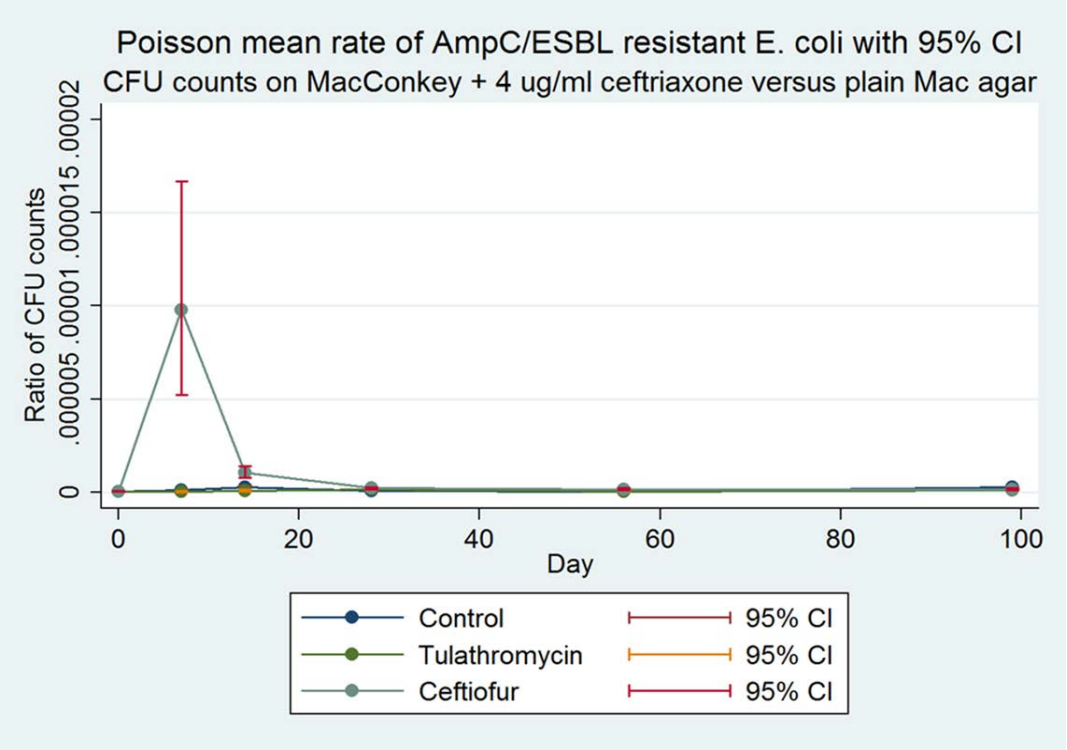

Figure 2.6. Poisson mean rate of AmpC and ESBL resistant E.coli