PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF *E. COLI* AND *SALMONELLA* SPP. IN MARKET SHOW SWINE AND CATTLE

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ABSTRACT

Fecal samples were collected from market hogs (n=82) and steers (n=84) at a statewide livestock show in Texas. Samples were analyzed to determine the prevalence and antimicrobial susceptibility of generic *Escherichia coli* and *Salmonella* spp. *Escherichia coli* populations were higher in hogs with an LSMean of 6.12 $\log_{10}$ CFU/g of feces compared to steer samples at 5.57 $\log_{10}$ CFU/g ($P<0.05$). *Salmonella* was more prevalent in hog fecal samples than steer samples ($P<0.05$) with 19.05% of hogs and 3.61% steers testing positive. Microbroth dilution plates were used to evaluate antimicrobial susceptibility. Market hog *E. coli* isolates (n=330) were resistant to Tetracycline and Sulfisoxazole with values of 96.67% and 69.70%; whereas steer isolates (n=332) were resistant to Tetracycline and Streptomycin with values of 55.12% and 32.53%, respectively. Market hog *Salmonella* isolates (n=18) were resistant to Tetracycline with a value of 77.78%; whereas steer isolates (n=5) were pansusceptible to all antimicrobials tested.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Livestock shows have played an important role for families in Texas since the establishment of the first statewide livestock show in 1886 (SFT, 2016). Livestock shows primarily consist of animal exhibits such as cattle, hogs, sheep, and goats. While these animals may be washed and cleaned on a daily basis (Texas Cooperative Extension, 2001), there are concerns with bacteria, such as *Escherichia coli* (*E. coli*) and *Salmonella*, that are often found in the feces of these animals (Keen et al., 2006; Croxen et al., 2013; Roug et al., 2013; Sahl et al., 2013). Many studies have indicated that bacteria can be spread through human contact with contaminated animal feces or surfaces around livestock due to proper hygiene practices not being utilized (Keen et al., 2006; Pabilonia et al., 2014). Moreover, the show animals that are designated as "market livestock" will eventually be introduced to the human food supply. Along with concerns of bacterial presence in feces which could lead to traditional foodborne illnesses, antibiotic resistant infection is becoming even more of a public concern. Bacteria have the ability to naturally evolve and resistance genes may be transferred in mobile genetic elements through horizontal gene transfer (MacGowen and Macnaughton, 2013; Brown-Jaque et al., 2015). Antibiotic resistance is a dynamic issue for both humans and animals. However, little research focuses on where market show animals fit into that dynamic. The objectives of this study were to: 1) determine the prevalence of generic *E. coli* and *Salmonella* spp. in the feces of market show hogs and steers, 2) determine the antimicrobial susceptibility profiles of both pathogens, and 3) determine differences of antibiotic resistance levels in bacteria from market swine and cattle.

LITERATURE REVIEW

Escherichia coli

Escherichia coli (*E. coli*) is a gram-negative, non-sporulating facultative anaerobe typically found in the gastrointestinal tract of humans and animals (Tenaillon et al., 2010; Sahl et al., 2013). *E. coli* is a member of the Enterobacteriaceae bacterial group. While many strains of *E. coli* are considered to be nonpathogenic, or commensal, *E. coli* has the ability to adapt and some strains may develop virulent genes (Wells et al., 2014). Bray (1945) is responsible for the first recognition of *E. coli* being pathogenic. Further research on *E. coli* has led to a greater understanding of the bacteria itself plus various strains. For instance, in humans there are several pathotypes of *E. coli* that are classified as enterovirulent. These strains are split into several categories such as enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroaggregative (EAggEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC), and diffusely adherent (DAEC) (CDC, 2015a; Wells et al., 2014). Enteroinvasive and enteroaggregative are both commonly associated with gastrointestinal (GI) disease in humans whereas enterotoxigenic, enteropathogenic, and enterohemorrhagic cause GI issues in not only humans but can also infect and colonize in other animals. In terms of food safety issues, EHEC is associated with producing shiga toxins (Kaper et al., 2004). Even though shiga-toxin producing *E. coli* (STEC) are primarily found in the gastrointestinal tract of ruminant animals, humans are at risk of infection via foodborne illnesses often obtained through meat products contaminated with feces containing STEC (Croxen et al., 2013). Likewise, E. *coli* O157:H7 is classified as an EHEC and STEC strain (Wells et al., 2014). This particular strain of *E. coli*, may lead to Hemorrhagic Colitis and Hemolytic Uremic Syndrome in humans;

while the latter may result in kidney failure, thereby leading to the death of infected individuals (Corrigan et al., 2001).

Commensal *Escherichia coli* strains are the non-pathogenic form of *E. coli*. Commensal strains can be found in the large intestine and they primarily dwell in the mucus layer covering the epithelial tissue of the digestive tract. The commensal strains will often be shed with the mucus passing through the tract and exit the body with fecal material (Tenaillon et al., 2010). Strains that are not located in the large intestine will survive off of nutrients found in the mucus (Chang et al., 2004). *Escherichia coli* is one of the first bacteria that will inhabit the gastrointestinal tract of humans when they are just mere infants. As humans reach the elderly age, the population of *E. coli* will begin to decrease (Penders et al., 2006). One of the benefits to having commensal *E. coli* in the large intestine is that they will often act to prevent other pathogenic bacteria from colonizing (Tenaillon, 2010).

Salmonella spp.

Salmonella spp. are considered to be a gram-negative bacillus (rod shaped) bacteria that live in the gastrointestinal tracts of both humans and animals (USDA-FSIS, 2013). *Salmonella* spp. are known to cause the foodborne infection Salmonellosis (USDA-FSIS, 2013; Callaway et al., 2008). Foodborne infections are a result of individuals eating foods that contain live pathogens which then grow in the intestines and cause illnesses. There are several types of food that, if contaminated, may aid in transferring *Salmonella* to humans. These foods include any meat product that is raw or undercooked, dairy products, vegetables, fruits, and even eggs (Maldonado et al., 2013; USDA-FSIS, 2013; Foley et al., 2008). Within the genus *Salmonella*, there are two species, *S. enterica* and *S. bongori*. *Salmonella enterica* is further divided into six subspecies that encompass over 2,500 unique serovars (Coburn et

al., 2007). Serovars within *S. enterica* are often associated with causing foodborne illness in both humans and animals. *Salmonella* Dublin, *Salmonella* Typhimurium, and *Salmonella* Choleraesuis are a few examples of serovars that occur in humans and animals, but each is host specific to a different animal. According to USDA-FSIS (2015), *S.* Enteritidis and *S.* Typhimurium are the two most commonly associated with human foodborne infections.

Show Animals and Pathogens of Importance

Cattle and hogs have served a dual purpose for many years. While some people might own them as commercial livestock, others may only use them for participating in livestock shows. Livestock shows have played an important role for families in Texas since the establishment of the first statewide livestock show in 1886 (SFT, 2016). Any livestock that are destined to be utilized as show animals are often maintained separately from the herd where they originally came from or in smaller group settings (Texas Cooperative Extension, 2001). Youth across Texas are required to complete training and assessment on good management practices, keeping food quality and safety as top priority, before they can validate their animals for eligibility to compete in a statewide show (TAMAE, 2016). Daily hygienic routines for market show livestock consist of rinsing or washing and cleaning the animals starting several months prior to show and throughout show times. It is highly encouraged that exhibitors maintain cleanliness of their animals and individual stalls or designated spot on the rail; plus walkways, pathways, or aisles where people and other animals may travel through.

Keen et al. (2006) performed a study that delved into the issue of the presence of Shiga-toxigenic *E. coli* O157:H7 at agricultural fairs in the United States. In 2002, 2,919 fecal samples were collected in two states at 29 county fairs, as well as three state fairs.

Results confirmed that STEC O157:H7 was prevalent in livestock at 31 of 32 fairs. Furthermore, cattle were the primary carriers with 1,407 (11.4% of samples) positive results. Prevalence in other species such as swine and sheep and goats were 1.2% of 1,102 and 3.6% of 364, respectively. Given the brief explanation of livestock exhibit management, it is vital to note that Keen et al. (2006) hypothesized that there would be lower STEC O157 levels in show animals versus commercially raised livestock. However, upon completion of their study, data demonstrated that prevalence of STEC O157:H7 in livestock show animals at fairs is similar to animals that were raised in commercial settings (e.g. feedlots). However, with proper sanitation practices, such as washing hands after touching livestock exhibits, the risk of humans contracting STEC O157, and other bacterial risks, can be decreased. *Salmonella* was detected on various environmental surfaces (feed, cages, tables, and floors) at poultry exhibits within county fairs (Pabilonia et al., 2014). Of those samples collected, they found that at least one sample was positive from 10 out of 11 fairs; with eleven different serovars detected after isolation. It was concluded that possible *Salmonella* contamination could be spread from the environmental surfaces to exhibitors, thereby causing the contamination to spread to other surfaces or people (Pabilonia et al., 2014). This particular study was conducted seven years after the *E. coli* study by Keen et al. (2006) whose samples were collected in 2002. It is evident that the recurring theme of utilizing proper hygiene at fairs continues to remain imperative.

Antibiotic Use

In humans, there are different antimicrobials that may be used to treat *E. coli* infections and Salmonellosis. According to Madappa and Hiong (2015), ampicillin and sulbactam (or cefoxitin) may be used to treat intra-abdominal abscesses from *E. coli* whereas

doxycycline, fluoroquinolononones, and rifaxmin can treat Traveler's Diarrhea (an enteric infection). *Salmonella* infections leading to Salmonellosis can be treated with ciprofloxacin, azithromycin, ceftriaxone, trimethoprim and sulfamethoxazole, and chloramphenicol (Klochko and Wallace, 2015).

For livestock, antimicrobials that are utilized in the form of injections have withdrawal periods. Withdrawal periods are defined as the number of days a producer must wait for the antibiotic residues to be out of the animal's system prior to being sent to slaughter, where its meat products will eventually be consumed, and even residues that may be in milk and eggs (McEwen and Fedorka-Cray, 2002). Treatment of various infections in cattle can include antibiotics such as amoxicillin, erythromycin, fluoroquinolone, penicillin, sulfonamides, and tylosin. In swine, amoxicillin, ampicillin, chlortetracyline, sulfamethazine, and tylosin can be used to treat various infections (McEwen and Fedorka-Cray, 2002). The Food and Drug Administration deemed antibiotic use to prevent, control, or treat certain diseases as judicious uses for antibiotics (FDA, 2012). Off-label use, such as using an antimicrobial to increase rate of gain in food animals, of the aforementioned drugs is considered to be injudicious and is prohibited (FDA, 2012; FDA, 2013).

Antimicrobial Resistance

Antimicrobial resistance (AMR) occurs when a microbe, pathogenic or nonpathogenic, demonstrates partial or full resistance to antimicrobials; after this occurs it is susceptible to absorbing genes that encode for AMR (Brown-Jaque et al., 2015; MacGowen and Macnaughton, 2013). Antimicrobial resistance genes can be carried on Mobile Genetic Elements (MGE) and can be transferred by Horizontal Gene Transfer (HGT) (Brown-Jaque et al., 2015; MacGowen and Macnaughton, 2013). The MGEs consist of plasmids and

transposons. Both MGE and HGT utilize conjugation, transduction, and transformation to transfer genes from host to host (Brown-Jaque et al., 2015; MacGowen and Macnaughton, 2013; Gogarten and Townsend, 2005). Conjugation occurs when there is direct cell to cell contact with plasmid transfer. Transduction is responsible for transferring bacterial DNA via bacteriophages and then those bacteriophages infect another host. Transformation occurs when naked AMR DNA is absorbed from the environment.

Current Applications in Agriculture Industry

Antimicrobial drugs can be used for many purposes in the agriculture industry. According to Viola and DeVincent (2006), antimicrobials can be used to treat illnesses ranging from skin to respiratory infections. Secondly, they can be used as a measure to prevent specific diseases during certain stages of animal production (also known as prophylactic treatment). Third, they can be used to treat large numbers of animals when there is a risk of disease spreading in the herd (also known as metaphylactic treatment). Lastly, they can be used to improve feed conversion efficiency or rate of gain for production purposes. When antimicrobials are used for production purposes, they are often administered at subtherapeutic levels (McEwen and Fedorka-Cray, 2002). According to Allen et al. (2013) subtherapeutic is defined as using a dosage that is lower than the dosage needed to actually treat a disease. The United States Food and Drug Administration recently published documents that outlined changes which essentially removed the option for utilizing antimicrobials as growth promoters (FDA, 2012). Given the many uses of antimicrobials, each time the antimicrobial is used it increases the selective pressure for bacteria to become resistant. To address concerns with antimicrobial resistance, several studies were conducted to evaluate how the administration of antimicrobials would affect antimicrobial resistance.

Two studies indicated that cattle, which have been administered antimicrobial drugs, have a higher likelihood of shedding antimicrobial resistant bacteria in the feces (Alexander et al., 2008; Sharma et al., 2008). Alexander et al. (2008) evaluated fecal samples from feedlot steers. Results indicated that the increase in prevalence of Tetracycline- and Ampicillin-resistant *E. coli* was largely due to the administration of Tetracycline with Sulfamethazine. A similar study conducted by Sharma et al. (2008) collected fecal samples from beef cattle that had been given either Chlortetracycline or Chlortetracycline with Sulfamethazine. Results demonstrated that fecal shedding of Tetracycline-resistant commensal *E. coli* was higher in cattle that received the combination of antimicrobials. Although the two previously mentioned studies saw increases in antimicrobial resistant bacteria due to the usage of certain antimicrobials, there are other studies that have results which conflict with their findings. For instance, in a study conducted by Kalmokoff et al. (2011), results indicated that antimicrobial resistant genes were present even in the absence of administered antimicrobials. This was especially evident in macrolide resistant genes being found in pigs that were not directly exposed to antimicrobials. Agga et al. (2015) also found tetracycline resistant genes in pigs that had not been directly exposed to antimicrobials. Antimicrobial resistance is a dynamic issue in that resistance genes can be found even if an antimicrobial hadn't been administered. Regardless, it is prudent for producers to utilize antimicrobials in a judicious manner (FDA, 2012).

Prevention of Antimicrobial Resistance

For several decades, many countries across the world have recognized that antimicrobial resistance is a growing problem that must be addressed accordingly. Food animal producers and major industry organizations alike are working towards being better

stewards of antibiotic use to prevent antimicrobial resistance. For instance, in the United States the Beef Checkoff Program has funded the Beef Quality Assurance (BQA) Program. This funding allows the BQA Program to provide materials and manuals for both producers and consumers on topics such as "A Beef Producers Guide for Judicious Use of Antimicrobials in Cattle" (Beef Quality Assurance, 2015). Also, the U.S. Centers for Disease Control and Prevention established the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) in 1996. This organization was specifically created to help monitor and investigate incidences reported by the CDC, Food and Drug Administration and United States Department of Agriculture (CDC, 2015b). Likewise, the Food and Drug Administration actively monitors the efficacy and safety of medically important antimicrobials that are used for both human and animal health. In 2003 the FDA published Guidance for Industry #152. This document largely focuses on maintaining the efficacy of medically important drugs, protecting the health of both humans and animals, and how to mitigate potential risk associated with antimicrobials (FDA, 2003). As a follow up, the FDA published Guidance for Industry #209 in April 2012. Guidance for Industry #209 brings forth the topic of judiciously using medically important antimicrobials. The FDA considers judicious use of antimicrobials beneficial in situations such as prevention, controlling, or treating certain diseases. The FDA thinks that the judicious use of medically important antimicrobials would motivate people to not use antibiotics for unnecessary or inappropriate situations and ultimately reduce the development of antimicrobial resistant bacteria (FDA, 2012). Also, in 2013 the FDA published Guidance for Industry #213. This guidance called for the voluntary elimination of using medically important antimicrobials as growth promoters in feed or water (FDA, 2013). This document also focuses on promoting increased

veterinary oversight in the initial decision making process on whether to use antimicrobials in feed or water. Drug sponsors would be able to voluntarily change product labels to remove terms such as “growth promotion” or “increase rate of gain” and add “include veterinary oversight” (FDA, 2013). As of January 1, 2017, antimicrobials may not be used in food or water for production purposes and a Veterinary Feed Directive must be obtained from a veterinarian in order to purchase them (FDA, 2013).

In 2010, World Organisation for Animal Health (OIE) formed a Tripartite Alliance with Food and Agriculture Organization of the United Nations (FAO), OIE, and World Health Organization (WHO). They firmly believe that tackling the antimicrobial resistance issue needs to be a joint effort between human health and animal health professionals. The goals of their alliance consist of: “1) ensure that antimicrobial agents maintain their efficacy, 2) promote the responsible and prudent use of these agents, and 3) enable access to high-quality medicines for all” (OIE, 2010). More recently, in May 2015, World Health Organization (2015a) proposed a Global Action Plan that would also aid in taking on the fight against antimicrobial resistance. Their plan includes five key goals such as: “1) improve awareness and understanding of antimicrobial resistance, 2) strengthen knowledge through surveillance and research, 3) reduce the incidence of infection, 4) optimize the use of antimicrobial agents, and 5) develop the economic case for sustainable investment that takes account of the needs of all countries, and increase investment in new medicines, diagnostic tools, vaccines and other interventions”.

Organizations in both the United States and Europe recognize that antimicrobial resistance is an ever growing problem that needs to be addressed accordingly. Efforts to

reduce or slow antimicrobial resistance should be met with a willingness to cooperate so that people may continue to utilize these drugs for their intended purpose.

MATERIALS AND METHODS

Sample Collection

Sample collection procedures used in this study were approved by the Angelo State University Institutional Animal Care and Use Committee (Appendix A, Form 16-01). Fecal samples were collected from eighty-four market steers (n=84) and market hogs (n=84) of varying breed types at a statewide livestock show in Texas. The fecal grab method, collecting off of fresh fecal pats, was used on both species. Portions of feces were collected from the top most area of fresh fecal pats to preserve the integrity of the sample. A fecal pat was deemed 'fresh' if it showed signs of moisture and no surface contamination. Only one sample was collected per stall to ensure no duplicate animals were tested. Approximately 50g of feces was collected using fresh sterile gloves and placed in a sterile collection cup, labeled with a unique identification number, and maintained in a cooler with ice packs until completion of sample collection. All samples were stored at 4°C in Angelo State University's Food Microbiology Laboratory and processed within 36 hours of collection.

Escherichia coli Enumeration and Isolation

Serial dilutions using Buffered Peptone Water were prepared and plated onto 3M *E. coli*/ Coliform Count Plate® (3M™ Petrifilm™, 2011) and incubated at 37±2°C for 24 hrs. *E. coli* populations were enumerated by counting colonies that were blue in color with associated gas bubbles as per manufacturer's guidelines. Four generic *E. coli* colonies were obtained using sterile disposable loops and isolated on MacConkey agar and incubated at 37±2°C for 24 hrs. Next, four isolated colonies were transferred to a 9 ml tube of Tryptic Soy Broth (TSB) and incubated at 37±2°C for 24 hrs. Isolate culture was combined with a sterile

1:1 glycerol solution (glycerine:deionized water) and stored in a -81°C freezer until antimicrobial susceptibility testing.

Salmonella Detection and Isolation

Salmonella spp. isolation was accomplished by selectively enriching one gram of feces in both 9ml of Tetrathionate (TT) broth and 9ml of Rappaport-Vassiliadis (RV) broth and incubated at 37±2°C for 24 hrs and 42±2°C for 24 hrs, respectively. Culture obtained from both broths was streaked onto Xylose Lysine Tergitol 4 (XLT4) plates and incubated at 37±2°C for 48 hrs. Samples were presumed positive if they exhibited colonies yellow to red in coloring with black centers (Difco-BD, Sparks, MD). Two presumptive positive isolates were utilized to inoculate test tubes of TSB and incubated at 37±2°C for 24 hrs. Isolate culture was combined with a sterile 1:1 glycerol solution (glycerine:deionized water) and stored in a -81°C freezer until further use. Final confirmation of presumptive positive isolates was conducted via latex agglutination. Upon thawing for resistance testing, the manufacturer's instructions of the Remel Colex Latex Agglutination Kit were followed and isolates were classified into a serogroup (Remel, Lenexa, KS). In brief, previously frozen *Salmonella* culture was streaked onto XLT4 and incubated at 37±2°C for 48 hrs. One well isolated colony was streaked onto Tryptic Soy Agar (TSA) and incubated for 37±2°C for 24 hrs. Next, two colonies were transferred from the TSA plate to a tube of sterile saline solution using a sterile disposable loop. The saline solution was then dispensed onto a manufacturer provided agglutination card and rotated for two minutes to allow for agglutination. Samples that were confirmed positive were utilized for antimicrobial susceptibility testing.

Antimicrobial Susceptibility Testing

Escherichia coli isolate culture was streaked onto MacConkey agar plates and incubated for 37±2°C for 24 hrs. One well isolated colony was then re-isolated on a TSA plate and incubated at 37±2°C for 18-24 hours. One colony was suspended in 4 ml of sterile, deionized water and vortexed. The homogenized solution was then adjusted to a 0.5 McFarland Polymer Turbidity Standard using a Thermo Scientific™ Sensititre™ Nephelometer (Thermo Scientific, Lenexa, KS). Ten µl of the suspended solution was transferred to 10 ml of Mueller-Hinton (MH) broth. The MH solution was vortexed and poured into a sterile seed trough. A multi-chamber pipette was used to transfer 50 µl of MH solution from the sterile seed trough to inoculate each of the 96 wells on the Sensititre® (CMV3AGNF) plate. Special care was taken to cover the Sensititre® plate with an adhesive seal while avoiding creases that would prevent proper sealing. The Sensititre® plates were incubated at 34-36°C for 18-24 hours. After incubation, the results were manually read using the Sensititre® Manual Viewer (Thermo Scientific). Wells that had a deposit of bacterial cells at the bottom of the plate, or cloudy growth throughout, were considered to be resistant to the amount of antimicrobial present in that well. Subsequently, the Minimum Inhibitory Concentration (MIC) of each antimicrobial was recorded as the lowest concentration of antimicrobial that inhibited visible growth. For this study, the Sensititre® plates included 14 antimicrobials of importance to humans and animals. Names and tested concentration levels of these antimicrobials are found in Table 1.

Antimicrobial susceptibility testing for *Salmonella* isolate culture was similar to that of *E. coli* except isolate culture was streaked onto XLT4 plates, incubated for 37±2°C for 48 hrs, and then transferred to TSA plates.

Table 1. Antimicrobial agent concentration and breakpoints

Antimicrobial Agent	**Concentration, µg/ml**	**MIC Breakpoint, µg/ml***
Amoxicillin-clavulanic acid	1-32	≥32/16
Ampicillin	1-32	≥32
Azithromycin	0.12-16	≥32
Cefoxitin	0.5-32	≥32
Ceftiofur	0.12-8	≥8
Ceftriaxone	0.25-64	≥4
Chloramphenicol	2-32	≥32
Ciprofloxacin		
Escherichia coli	0.015-4	≥4
Salmonella spp.	0.015-4	≥1
Gentamicin	0.25-16	≥16
Nalidixic Acid	0.5-32	≥32
Streptomycin	2-64	≥32
Sulfisoxazole	16-256	≥512
Tetracycline	4-32	≥16
Trimethoprim/Sulfamethoxazole	0.12/2.38-4/76	≥4/76

*MIC Breakpoint = Minimum Inhibitory Concentration Breakpoint; obtained from the National Antimicrobial Resistance Monitoring System's 2014 Human Isolates Surveillance Report.

Statistical Analysis

Various procedures of SAS (Cary, NC; Version 9.1.3) were used to compile descriptive statistics. *Escherichia coli* populations were transformed to a log base 10. Significant differences between *E. coli* populations from market show hogs and steers were evaluated using mixed procedures (PROC MIXED) of SAS. Chi square analysis was used to determine differences in prevalence of *Salmonella* between animal species. Isolates were classified as resistant or susceptible to each antimicrobial using established breakpoints provided by the National Antimicrobial Resistance Monitoring Service (Table 1; NARMS, 2014). Minimum Inhibitory Concentrations (MIC) were transformed to a log base 2. Differences in MIC of each antimicrobial drug between animal species were also analyzed using the mixed procedures of SAS. Chi Square Analysis was utilized to evaluate differences in frequency of resistant versus susceptible isolates. All significant differences were evaluated at a predetermined $\alpha \leq 0.05$.

RESULTS & DISCUSSION

Escherichia coli

Fecal samples were analyzed for presence and population level of generic *Escherichia coli*. Out of 168 total fecal samples collected, 97.60% of hogs (82 of 84) were positive for generic *E. coli*, whereas 100% of steers (n=84) were positive. *Escherichia coli* populations were higher in hogs with an Least Squares Means (LSMeans) of 6.12 $\log_{10}$ CFU/g of feces compared to steer samples at 5.57 $\log_{10}$ CFU/g ($P<0.05$).

Fourteen antimicrobials were evaluated for antimicrobial susceptibility testing of generic *E. coli* isolates obtained from market show hogs and steers. Of the 662 total generic *E. coli* isolates utilized for antimicrobial susceptibility testing, 98.18% (324 of 330 tested) of hog isolates and 63.25% (210 of 332 tested) of steer isolates exhibited resistance to at least one of the 14 antimicrobials tested. Additionally, it is important to consider what percentage of isolates that exhibited resistance to more than one antimicrobial drug. Of the 14 antimicrobials that were tested in the panel, 85.16% (281 of 330 tested) market hog isolates exhibited resistance to two antimicrobials or more (Figure 1). When evaluating market steer isolates, only 36.73% (122 of 332 tested) of isolates were resistant to two antimicrobials or more. When evaluating the different resistance patterns shown by the isolates, there were 87 unique resistance patterns which were exhibited by the *E. coli* isolates from all livestock tested. In market hog isolates, the most common pattern was resistance to Tetracycline alone in 11.82% of all tested isolates (Table 2). Resistance patterns were also observed for both

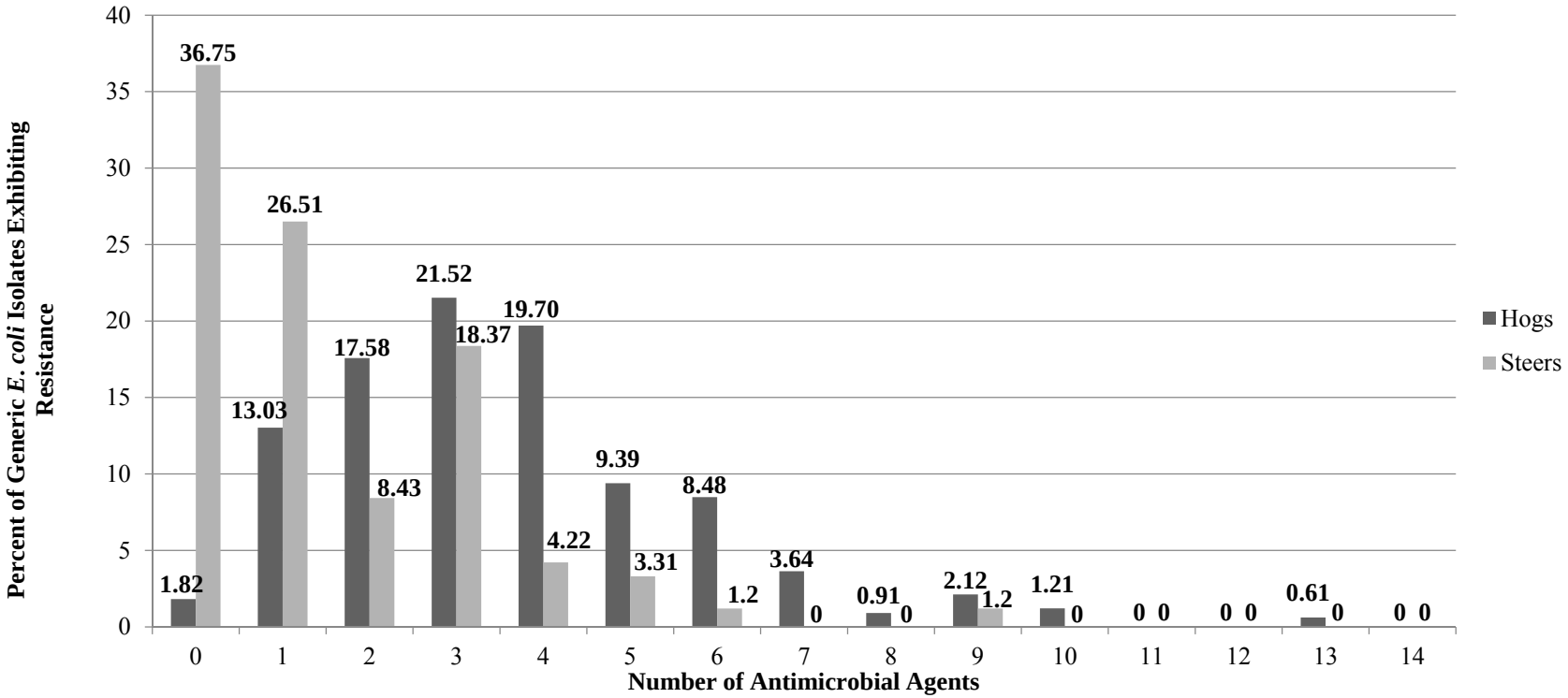

Figure 1. Percentage of generic *Escherichia coli* isolates obtained from market hogs (n=330) and steers (n=332) based on the number of antimicrobial drugs to which resistance was exhibited.

Table 2. Most frequent antimicrobial drug resistance patterns of *Escherichia coli* isolates obtained from market show hogs (n=330) and steers (n=332)

Species	Percent Isolates Resistant	Isolate Frequency	Number of Drugs Resistant	Antimicrobials to Which Isolates were Resistant
Market Hogs	11.82	39	1	Tetracycline
	8.18	27	2	Streptomycin, Tetracycline
	7.58	25	2	Sulfisoxazole, Tetracycline
	7.27	24	4	Chloramphenicol, Sulfisoxazole, Streptomycin, Tetracycline
	7.27	24	3	Ampicillin, Sulfisoxazole, Tetracycline
Market Steers	36.75	122	0	Pansusceptible*
	21.39	71	1	Tetracycline
	15.36	51	3	Sulfisoxazole, Streptomycin, Tetracycline
	3.92	13	4	Chloramphenicol, Sulfisoxazole, Streptomycin, Tetracycline
	3.31	11	2	Streptomycin, Tetracycline

*Pansusceptible=susceptible to all antimicrobial drugs tested

Streptomycin and Tetracycline in 8.18% of hog isolates. As for market steer isolates, the most common pattern of resistance was seen in 36.75% of isolates exhibiting pansusceptibilty to all 14 antimicrobial drugs tested. Also, 21.39% of market steer isolates had a resistance pattern to Tetracycline alone (Table 2). In a similar study conducted by Roug et al. (2013), fecal samples were collected from pigs and dairy cattle at a county fair in California and screened for the presence of commensal *Escherichia coli*. Ten isolates were obtained from both species and utilized for antimicrobial resistance testing. Out of the ten pig isolates utilized, the most common resistance pattern exhibited was to Tetracycline as it was present in all of the patterns. Resistance patterns were also exhibited to Sulfisoxazole, Streptomycin, and Chloramphenicol in nine, four, and five of the patterns, respectively. For dairy cattle isolates, the most common pattern was to Tetracycline as it was present in three patterns (Roug et al., 2013). Similarities were identified in the results from the current study and Roug et al. (2013). Notably, the most common resistance pattern exhibited in swine isolates from both studies was to Tetracycline. For cattle isolates from both studies, there was similar resistance patterns exhibited to Tetracycline as well. Although the current study and Roug et al. (2013) were not conducted in the same geographic location, results from both studies were based on similar species which provides a common ground for results to be compared. For this particular research avenue, it would be beneficial to conduct similar studies in other states to see if geographic location plays a role in antimicrobial susceptibility of generic *Escherichia coli* isolates obtained from show animals.

During antimicrobial susceptibility testing, minimum inhibitory concentration (MIC) breakpoints, provided by the National Antimicrobial Resistance Monitoring System, were utilized to determine if bacteria were resistant or susceptible to each of the antimicrobials

(NARMS, 2014). When evaluating resistance to the various antimicrobials tested, isolates from market hogs exhibited the most common resistance to Tetracycline, Sulfisoxazole, and Streptomycin with 96.67%, 69.70%, and 53.64% of isolates exhibiting resistance to the respective antimicrobial (Figure 2). Isolates from market steers exhibited the most common resistance to Tetracycline, Streptomycin, and Sulfisoxazole with 55.12%, 32.53%, and 28.61% of isolates exhibiting resistance, respectively (Figure 2). According to the Roug et al. (2013) study, out of all commensal *E. coli* isolates utilized for antimicrobial susceptibility testing, isolates from pigs demonstrated resistance to Tetracycline (10 out of 10 isolates), Sulfisoxazole (9 out of 10 isolates), and Ampicillin, Chloramphenicol, Kanamycin, and Streptomycin (5 out of 10 isolates). Commensal *Escherichia coli* isolates from cattle in their study also demonstrated resistance to Tetracycline (3 out of 10 isolates), Sulfisoxazole (2 out of 10 isolates), and Streptomycin, Kanamycin, and Ampicillin (1 out of 10 isolates). Roug et al. (2013) also reported that none of the *E. coli* isolates were resistant to Amoxicillin/Clavulanic Acid, Cefoxitin, Ceftiofur, Ciprofloxacin, Gentamicin, and Nalidixic Acid. Results from Roug et al. (2013) align with the current study in that more hog isolates, when compared to steers/dairy cattle, exhibited resistance to Tetracycline, Sulfisoxazole, Ampicillin, Chloramphenicol, and Streptomycin. With livestock designated for show being maintained differently than those that are commercially raised, it is important to evaluate similarities and differences between market show livestock and commercially raised livestock in terms of antimicrobial resistance. In a study conducted by Jacob et al. (2008),

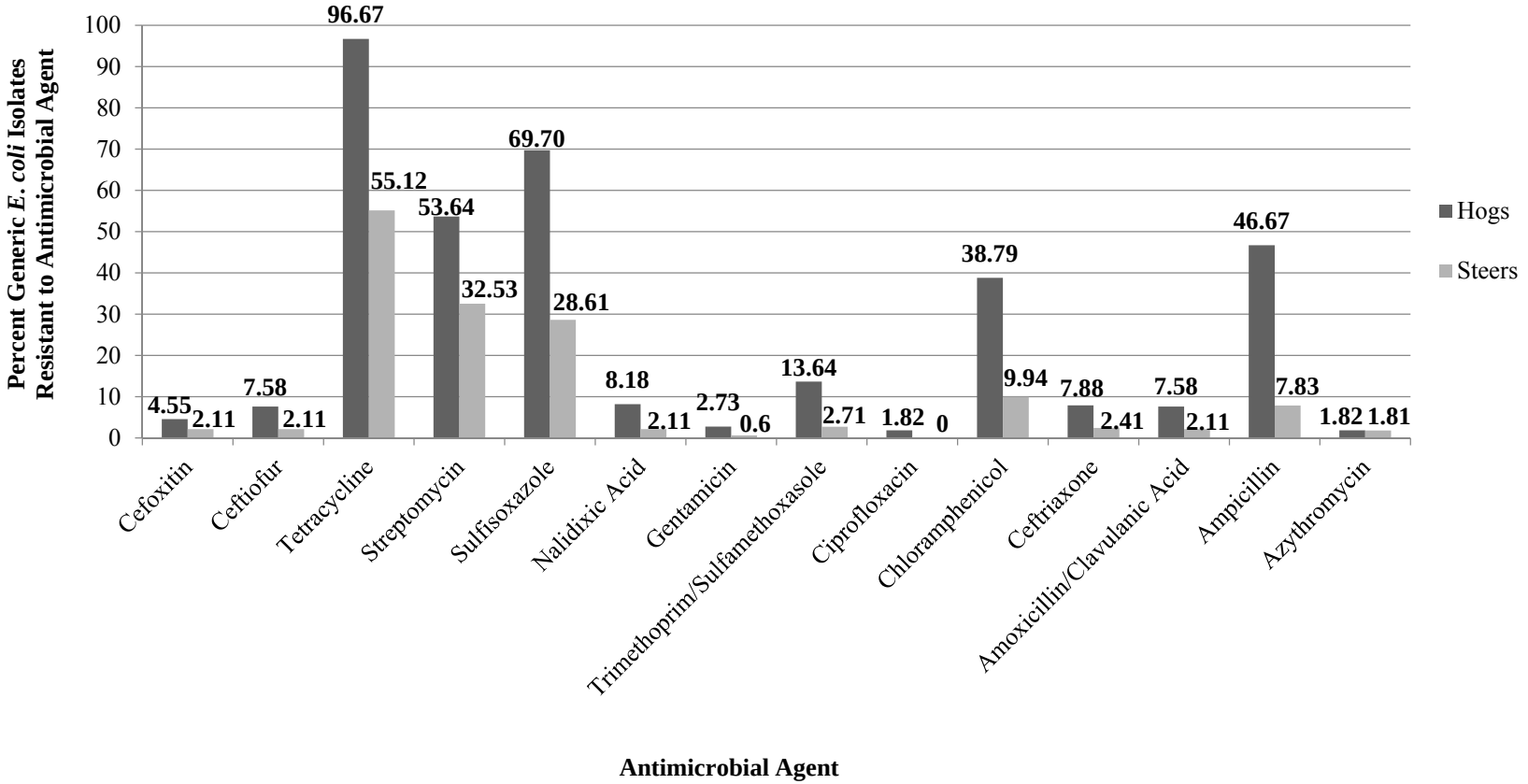

Figure 2. Percentage of generic *Escherichia coli* isolates from market hogs (n=330) and steers (n=332) resistant to various antimicrobial drugs.

188 generic *E. coli* isolates were obtained from feedlot heifers that were fed a diet of wet corn distillers grains with or without the addition of Monensin and Tylosin. Antimicrobial susceptibility testing of those isolates revealed that majority were susceptible to Aminoglycosides. The current study tested both Gentamicin and Streptomycin which fall under the Aminoglycoside antimicrobial class (NARMS, 2014). Notably, results from the current study indicated that market show steer isolates exhibited resistance to both Streptomycin and Gentamicin with values of 32.53% and 0.6%, respectively. The current study did not have access to the diet history of livestock in which fecal samples were collected from; it would be beneficial to have that type of information in future research evaluating antimicrobial susceptibility of show animals.

Table 3 shows the percentage of all market show hog *E. coli* isolates (n=330) tested based on the percent that fell within the various minimum inhibitory concentrations (MIC) for each antimicrobial drug on the panel. The largest percentage of isolates exhibited MICs at the highest concentration tested for Tetracycline, Ampicillin, Streptomycin, and Chloramphenicol with values of 92.73%, 45.45%, 28.18%, and 21.21%, respectively. Results from the Jacob et al. (2008) study indicate that none of the generic *E. coli* isolates obtained from feedlot heifers were resistant to Danofloxacin or Enrofloxacin (which are classified as Quinolones). In the current study, of the 330 hog isolates tested, 96% exhibited MICs of 0.25 µg/ml or less in Ciprofloxacin; which falls into the Quinolone class of drugs. This is notably less than the resistant breakpoint of 4 µg/ml. This result is encouraging as Ciprofloxacin is important in treating various infections and has been identified by World Health Organization as clinically important (WHO, 2015b). Ceftriaxone, Trimethoprim/

Table 3. Percentage of generic *Escherichia coli* isolates obtained from market show hogs (n= 330) on the basis of Minimum Inhibitory Concentration (MIC) ratio

	MIC Ratio[1]										
Antimicrobial	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Lowest Concentration Tested, µg/ml**
Cefoxitin	0.00	0.00	5.45	49.09	35.76	5.15	0.91	3.64	-	-	0.5
Azithromycin[2]	0.00	0.00	0.00	1.21	5.76	49.70	36.06	5.45	1.82	-	0.12
Chloramphenicol	2.42	10.30	42.12	6.36	17.58	21.21	-	-	-	-	2.0
Tetracycline	2.42	0.91	0.00	3.94	92.73	-	-	-	-	-	4.0
Ceftriaxone	81.21	5.76	4.24	0.91	0.30	0.00	1.21	1.21	1.21	3.94	0.25
Amoxicillin/Clavulanic Acid	2.12	24.24	41.82	21.52	2.73	6.67	0.91	-	-	-	1.0/0.5
Ciprofloxacin	60.00	9.09	2.42	0.61	4.55	19.39	0.91	1.21	0.30	1.52	0.015
Gentamicin	22.73	53.64	11.82	5.15	3.03	0.91	0.61	2.12	-	-	0.25
Nalidixic Acid	0.00	0.91	42.12	23.03	6.06	19.70	6.36	1.82	-	-	0.5
Ceftiofur	0.30	29.09	55.15	5.76	1.21	0.91	0.91	6.67	-	-	0.12
Sulfisoxazole[2]	18.79	9.39	1.82	0.00	0.30	69.70	-	-	-	-	16.0
Trimethoprim/Sulfamethoxazole	63.33	16.67	4.85	1.21	0.30	0.00	13.64	-	-	-	0.12
Ampicillin	5.45	32.12	10.61	4.85	0.30	1.21	45.45	-	-	-	1.0
Streptomycin	0.61	16.36	18.18	11.21	14.85	10.61	28.18	-	-	-	2.0

[1] Ratios were obtained by dividing each MIC by the lowest concentration on the specific antimicrobial tested. The ratio was then turned into a log base 2 for comparison.

[2] Highlighted dash marks indicate breakpoint not on Sensititre® panel

- = Value greater than the highest number tested on the panel

*Shaded values represent isolates considered resistant for individual drugs based on breakpoints established by the National Antimicrobial Resistance Monitoring System (NARMS).

Sulfamethoxazole, Ciprofloxacin, and Sulfisoxazole had the largest percentage of isolates exhibiting MICs at the lowest concentration tested with values of 81.21%, 63.33%, 60.00%, and 18.79%, respectively. Table 4 displays the percentage of all market show steer *E. coli* isolates (n=332) tested based on the percent that fall within the various MICs for each antimicrobial. Tetracycline was the only antimicrobial that displayed the largest percentage of isolates exhibiting MICs at the highest concentration tested with a value of 44.28%. Additionally, Tetracycline exhibited 37.65% of isolates that had MICs at the lowest concentration tested; which leaves the remaining 50% of isolates resistant. Ceftriaxone, Trimethoprim/Sulfamethoxazole, Ciprofloxacin, and Sulfisoxazole had the largest percentage of isolates exhibiting MICs at the lowest concentration tested with values of 91.87%, 88.55%, 73.49%, and 54.52%, respectively. It is important to note that Ciprofloxacin did not have any isolates with MICs in the four highest concentration levels tested and 97.6% of isolates had MICs at or below 0.06 µg/ml. Medically, bacteria with MICs that fall well below the breakpoint are more likely to be controlled by the prescribed drug.

Lastly, the differences between LSMeans of generic *E. coli* from market show hogs and steers were evaluated on the basis of MICs for antimicrobial agents tested (Table 5). Market hog isolates displayed higher MICs in 13 out of 14 tested antimicrobials when compared to steer isolates. The most notable differences in LSMeans were observed in Sulfisoxazole, Ampicillin, Tetracycline, and Ciprofloxacin. Market hog isolates had an LSMean of 7.62 $\log_2$ µg/ml for Sulfisoxazole and steers had 5.62 $\log_2$ µg/ml ($P<0.0001$).

Table 4. Percentage of generic *Escherichia coli* isolates obtained from market show steers (n=332) on the basis of Minimum Inhibitory Concentration (MIC) ratio

	MIC Ratio[1]										
Antimicrobial	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Lowest Concentration Tested, µg/ml**
Cefoxitin	0.00	0.60	9.34	50.90	32.23	4.82	0.30	1.81	-	-	0.5
Azithromycin[2]	0.00	0.00	0.00	0.60	10.24	57.23	27.11	3.01	1.81	-	0.12
Chloramphenicol	1.51	16.87	65.96	5.72	1.81	8.13	-	-	-	-	2.0
Tetracycline	37.65	7.23	5.12	5.72	44.28	-	-	-	-	-	4.0
Ceftriaxone	91.87	4.22	1.51	0.00	0.30	0.60	0.00	1.20	0.00	0.30	0.25
Amoxicillin/Clavulanic Acid	8.43	31.93	50.30	6.02	1.20	1.20	0.90	-	-	-	1.0/0.5
Ciprofloxacin	73.49	18.98	2.71	1.81	2.41	0.60	0.00	0.00	0.00	0.00	0.015
Gentamicin	13.25	65.96	15.96	3.31	0.90	0.00	0.30	0.30	-	-	0.25
Naladixic Acid	0.00	1.81	55.72	34.34	4.22	1.81	0.00	2.11	-	-	0.5
Ceftiofur	4.52	30.12	54.22	2.71	5.72	0.60	0.90	1.20	-	-	0.12
Sulfisoxazole[2]	54.52	14.46	2.11	0.30	0.00	28.61	-	-	-	-	16.0
Trimethoprim/Sulfamethoxazole	88.55	6.93	1.20	0.60	0.00	0.30	2.41	-	-	-	0.12
Ampicillin	12.35	57.53	18.37	2.41	1.51	1.20	6.63	-	-	-	1.0
Streptomycin	2.71	21.69	36.45	6.63	7.83	8.43	16.27	-	-	-	2.0

[1] Ratios were obtained by dividing each MIC by the lowest concentration on the specific antimicrobial tested. The ratio was then turned into a log base 2 for comparison.

[2] Highlighted dash marks indicate breakpoint not on Sensititre® panel

- = Value greater than the highest number tested on the panel

*Shaded values represent isolates considered resistant for individual drugs based on breakpoints established by the National Antimicrobial Resistance Monitoring System (NARMS).

Table 5. Least Squares Means of generic *Escherichia coli* of Minimum Inhibitory Concentrations (MIC) (Log_2 µg/ml) for antimicrobial agents tested

	Market Hogs (n=300)		Market Steers (n=332)		
Antimicrobial Drug	**MIC, µg/ml**	**SE***	**MIC, µg/ml**	**SE**	***P*-value**[a]
Cefoxitin	2.57	0.05	2.39	0.05	0.01
Azithromycin	2.44	0.04	2.27	0.04	0.00
Chloramphenicol	3.90	0.06	3.13	0.06	<0.0001
Tetracycline	5.83	0.07	4.11	0.07	<0.0001
Ceftriaxone	-1.20	0.09	-1.77	0.09	<0.0001
Amoxicillin/clavulanic acid	2.22	0.05	1.66	0.05	<0.0001
Ciprofloxacin	-4.43	0.09	-5.63	0.09	<0.0001
Gentamicin	-0.72	0.06	-0.84	0.06	0.14
Nalidixic Acid	2.27	0.06	1.56	0.06	<0.0001
Ceftiofur	-0.81	0.07	-1.13	0.07	<0.05
Sulfisoxazole	7.62	0.11	5.62	0.11	<0.0001
Trimethoprim/sulfamethoxazole	-1.90	0.08	-2.78	0.08	<0.0001
Ampicillin	3.47	0.10	1.53	0.10	<0.0001
Streptomycin	4.67	0.10	3.85	0.10	<0.0001

[a] MIC values within an antimicrobial that have $P \leq 0.05$ differ

* SE= Standard Error

Additionally, market hog isolates had an LSMean of 3.47 $\log_2$ µg/ml for Ampicillin whereas steer isolates had 1.53 $\log_2$ µg/ml (P<0.0001). Market hog isolates had an LSMean of 5.83 $\log_2$ µg/ml for Tetracycline and steer isolates had 4.11 $\log_2$ µg/ml (P<0.0001). When evaluating Ciprofloxacin, hog isolates had an LSMean of -4.43 $\log_2$ µg/ml and steers had -5.63 $\log_2$ µg/ml (P<0.0001).

The current study did not obtain health or diet records prior to fecal sample collection; therefore, a definitive statement identifying the factors that contributed to the antimicrobial resistance of generic *E. coli* isolates obtained cannot be made. Although generic *E. coli* is considered to be non-pathogenic, it still has the potential to gain and transfer antimicrobial resistant genes to other bacteria; therefore, it is equally important to monitor antimicrobial susceptibility within this group of bacteria (Wells et al., 2014).

***Salmonella* spp.**

Fecal samples obtained from market show hogs and steers were analyzed to determine the prevalence of *Salmonella*. *Salmonella* was more prevalent in hog samples than steer samples (P<0.05) with 19.05% of hogs (16 of 84) and 3.61% steers (3 of 83) testing positive. Upon completion of agglutination testing, *Salmonella* isolates confirmed positive were placed into one of three serogroups. Of the market show hog isolates obtained, 88.88% (16 out of 18) were classified into serogroup B whereas 11.11% (2 out of 18) were classified into serogroup E or G. Out of the market steer isolates obtained, 40% (2 out of 5) were classified in serogroup B, 40% in serogroup E or G, and 20% (1 out of 5) were classified in serogroup C. Since *Salmonella* spp. isolates were not serotyped in this study, examples of potential serotypes and their respective serogroup will be discussed. According to CDC

compiled statistics from 1968-2011, *S.*Typhimurium, *S*. Heidelberg, and *S*. Derby fall under serogroup B. Out of 52,889 non-human *S*. Heidelberg isolates collected between 1968-2011, 2.11% of those were from bovine and 1.55% from porcine (CDC, 2013). Of the 13,958 non-human *S*. Derby isolates, 3.29% were from bovine and 66.89% were from porcine. Serotype *S.* Anatum falls under serogroup E . Out of the 16,227 *S.* Anatum isolates, 19.50% were from bovine and 13.83% from porcine. *S*. Montevideo and *S*. Newport fall under serogroup C. Of the 18,245 *S*. Montevideo isolates collected, 25.60% were from bovine and 1.10% from porcine. Lastly, of the 14,811 *S*. Newport isolates collected, 49.27% were from bovine and 2.58% from hogs. Roug et al. (2013) conducted a study in California in which fecal samples were collected from dairy cattle and pigs at a county fair. None of the fecal samples collected from dairy cattle (16 tested), sheep (35 tested), or goats (11 tested) tested positive for *Salmonella*. However, positive fecal samples were obtained from pigs (7 of 31 tested). Two *Salmonella* Derby isolates were obtained from pigs. In a study conducted by Pabilonia et al. (2014), *Salmonella* was detected on various environmental surfaces (feed, cages, tables, and floors) at poultry exhibits within county fairs in Colorado. Of those samples collected, they found that at least one sample was positive from 10 out of 11 fairs. Out of all samples collected (n=55), *Salmonella* spp. isolates were obtained from 28 samples (50.9%). According to CDC (2017) surveillance data, all aforementioned serotypes, with the exception of *S*. Anatum, have been linked with foodborne illnesses in humans since 2014.

Salmonella isolates obtained from fecal samples of market show hogs and steers were utilized for antimicrobial susceptibility testing. Of the 18 *Salmonella* isolates obtained from market hogs, 83.33% exhibited resistance to at least one antimicrobial. It is also important to consider the percentage of isolates that exhibited resistance based on the number of

antimicrobial drugs tested. Of the 14 antimicrobials that were tested in the panel, 55.56% (10 out of 18 tested) market hog isolates exhibited resistance to two or more antimicrobial agents (Table 6). However, market steer isolates exhibited pansusceptibility to all tested antimicrobials. Along with analysis of the percent to which resistance was exhibited for a number of antimicrobials, there were 10 unique resistance patterns which were exhibited by the 23 isolates collected from all livestock. In market hog isolates, a resistance pattern was observed with 22.22% isolates resistant to Tetracycline alone and 22.22% resistant to Ampicillin, Sulfisoxazole, Streptomycin, and Tetracycline (Table 7). The remainder (16.67%) were pansusceptible. Market steers exhibited 100% (5 isolates) pansusceptibilty.

When evaluating resistance to the various antimicrobials, isolates from market hogs (n=18) exhibited the most common resistance to Tetracycline, Streptomycin, and Sulfisoxazole with 77.78%, 44.44% and 44.44% of isolates resistant to the respective antimicrobial (Figure 3). None of the five *Salmonella* isolates from market steers exhibited clinical resistance to any of the tested antimicrobials. In the study conducted by Roug et al. (2013), two *Salmonella* Derby isolates were obtained from pigs. Those isolates were utilized for antimicrobial susceptibility testing and were resistant to Florfenicol, Spectinomycin, Sulphachloropyridazine, Sulphadimethoxime, Sulphathizole, Chlortetracycline, and Oxytetracycline. According to Roug et al. (2013) none of the strains were resistant to Ampicillin, Ceftiofur, Gentamicin, or Trimethoprim/Sulphamethoxazole. The results from Roug et al. (2013) are similar to results from the current study in which Tetracycline was the most common antimicrobial to which isolates exhibited resistance.

Table 6. Percentage of *Salmonella* spp. isolates based on the number of antimicrobial drugs to which resistance was exhibited

# Antimicrobial Agents	% Market Hogs (n=18)	% Market Steers (n=5)
0	16.7	100.00
1	27.78	0.00
2	11.11	0.00
3	0.00	0.00
4	27.78	0.00
5	11.11	0.00
6	0.00	0.00
7	0.00	0.00
8	0.00	0.00
9	5.56	0.00
10	0.00	0.00
11	0.00	0.00
12	0.00	0.00
13	0.00	0.00
14	0.00	0.00

Table 7. Most frequent antimicrobial drug resistance patterns of *Salmonella* spp. isolates obtained from market show hogs (n=18) and steers (n=5)

Species	Percent Isolates Resistant (Frequency)	Number of Drugs Resistant	Antimicrobials to Which Isolates were Resistant
Market Hogs	22.22 (4)	1	Tetracycline
	22.22 (4)	4	Ampicillin, Sulfisoxazole, Streptomycin, Tetracycline
	16.67 (3)	0	Pansusceptible*
Market Steers	100.00 (5)	0	Pansusceptible*

*Pansusceptible=susceptible to all antimicrobial drugs tested

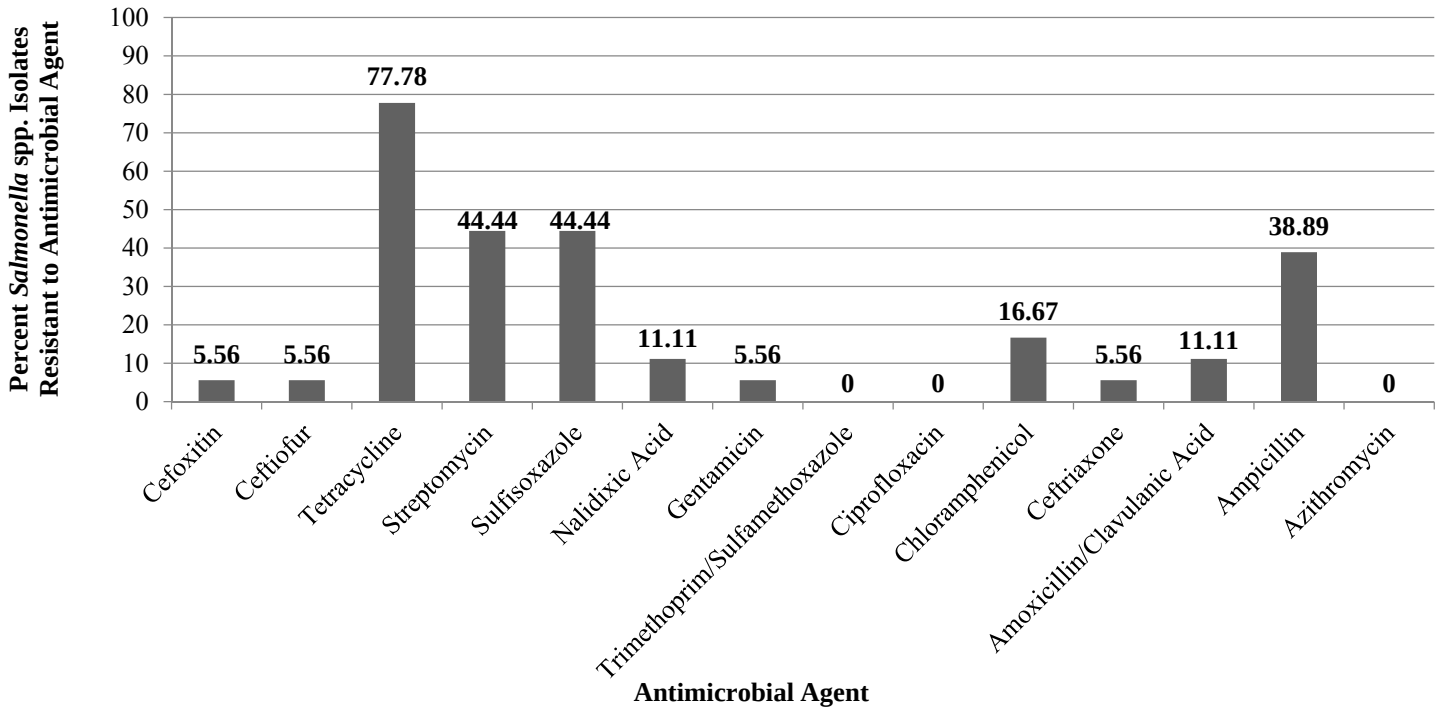

Figure 3. Percentage of *Salmonella* spp. isolates from market hogs (n=18) resistant to various antimicrobial drugs.

Table 8 shows the percentage of all market show hog *Salmonella* spp. isolates (n=23) tested based on the minimum inhibitory concentration (MIC) for each antimicrobial drug on the panel. The largest percentage of isolates exhibited MICs at the highest concentration tested for Tetracycline and Streptomycin with 72.22% and 44.44%, respectively. However, Ceftriaxone, Ciprofloxacin, Gentamicin, Nalidixic Acid, and Trimethoprim/ Sulfamethoxazole did not have any isolates exhibit MICs at the highest tested concentration. Instead, Ceftriaxone, Trimethoprim/Sulfamethoxazole, Amoxicillin/Clavulanic Acid, Ampicillin, Gentamicin, and Ciprofloxacin had the largest percentage of isolates exhibiting MICs at the lowest level tested with values of 88.89%, 88.89%, 61.11%, 61.11%, 55.56%, and 44.44%, respectively. Ciprofloxacin had results similar to those seen in *E. coli*. Table 9 displays the percentage of all market show steer *E. coli* isolates (n=5) tested based on the MIC for each antimicrobial. No antimicrobials tested had any isolates that exhibited MICs in the highest concentration tested. Given the small and unequal sample size, basic mean minimum inhibitory concentrations (MIC) for the antimicrobial agents tested are presented in Table 10. When compared to market steer isolates, market hogs had consistently higher numerical means MICs. However, true statistical differences were not assessed. Market hogs had a mean MIC of 5.05 $\log_2$ CFU/g for Tetracycline and steer isolates had 2.00 $\log_2$ µg/ml. Also, market hog isolates exhibited mean MIC of 2.33 $\log_2$ µg/ml for Ampicillin, while steer isolates had a mean MIC of 0.00 $\log_2$ µg/ml. Next, market hogs exhibited a mean MIC of 4.88 $\log_2$ µg/ml for Streptomycin, whereas steers had a mean MIC of 3.40 $\log_2$ µg/ml. Amoxicillin/Clavulanic Acid displayed a mean MIC of 1.27 $\log_2$ µg/ml for market hog

Table 8. Percentage of *Salmonella* spp. isolates collected from market show hogs (n=18) on the basis of Minimum Inhibitory Concentration (MIC) ratio

	MIC Ratio[1]										
Antimicrobial	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Lowest Concentration Tested, µg/ml**
Cefoxitin	0.00	0.00	38.89	38.89	16.67	0.00	0.00	5.56	-	-	0.5
Azithromycin[2]	0.00	0.00	0.00	0.00	0.00	72.22	27.78	0.00	0.00	-	0.12
Chloramphenicol	0.00	22.22	61.11	0.00	5.56	11.11	-	-	-	-	2.0
Tetracycline	22.22	0.00	0.00	5.56	72.22	-	-	-	-	-	4.0
Ceftriaxone	88.89	0.00	5.56	0.00	0.00	0.00	0.00	5.56	0.00	0.00	0.25
Amoxicillin/Clavulanic Acid	61.11	0.00	22.22	5.56	0.00	0.00	11.11	-	-	-	1.0/0.5
Ciprofloxacin	44.44	44.44	5.56	0.00	0.00	5.56	0.00	0.00	0.00	0.00	0.015
Gentamicin	55.56	21.78	11.11	0.00	0.00	0.00	5.56	0.00	-	-	0.25
Nalidixic Acid	0.00	0.00	33.33	55.56	0.00	0.00	11.11	0.00	-	-	0.5
Ceftiofur	0.00	0.00	5.56	77.78	5.56	5.56	0.00	5.56	-	-	0.12
Sulfisoxazole[2]	5.56	27.78	11.11	5.56	5.56	44.44	-	-	-	-	16.0
Trimethoprim/Sulfamethoxazole	88.89	11.11	0.00	0.00	0.00	0.00	0.00	-	-	-	0.12
Ampicillin	61.11	0.00	0.00	0.00	0.00	0.00	38.89	-	-	-	1.0
Streptomycin	0.00	11.11	22.22	22.22	0.00	0.00	44.44	-	-	-	2.0

[1] Ratios were obtained by dividing each MIC by the lowest concentration on the specific antimicrobial tested. The ratio was then turned into a log base 2 for comparison.

[2] Highlighted dash marks indicate breakpoint not on Sensititre® panel

- = Value greater than the highest number tested on the panel

*Shaded values represent isolates considered resistant for individual drugs based on breakpoints established by the National Antimicrobial Resistance Monitoring System (NARMS).

Table 9. Percentage of *Salmonella* spp. isolates collected from market show steers (n=5) on the basis of Minimum Inhibitory Concentration (MIC) ratio

	MIC Ratio[1]										
Antimicrobial	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Lowest Concentration Tested, µg/ml**
Cefoxitin	0.00	0.00	60.00	40.00	0.00	0.00	0.00	0.00	-	-	0.5
Azithromycin[2]	0.00	0.00	0.00	0.00	0.00	80.00	20.00	0.00	0.00	-	0.12
Chloramphenicol	20.00	60.00	20.00	0.00	0.00	0.00	-	-	-	-	2.0
Tetracycline	100.00	0.00	0.00	0.00	0.00	-	-	-	-	-	4.0
Ceftriaxone	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25
Amoxicillin/Clavulanic Acid	100.00	0.00	0.00	0.00	0.00	0.00	0.00	-	-	-	1.0/0.5
Ciprofloxacin	80.00	20.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.015
Gentamicin	100.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	-	0.25
Nalidixic Acid	0.00	0.00	20.00	80.00	0.00	0.00	0.00	0.00	-	-	0.5
Ceftiofur	0.00	0.00	40.00	40.00	20.00	0.00	0.00	0.00	-	-	0.12
Sulfisoxazole[2]	0.00	0.00	80.00	0.00	20.00	0.00	-	-	-	-	16.0
Trimethoprim/Sulfamethoxazole	80.00	0.00	0.00	20.00	0.00	0.00	0.00	-	-	-	0.12
Ampicillin	100.00	0.00	0.00	0.00	0.00	0.00	0.00	-	-	-	1.0
Streptomycin	0.00	0.00	60.00	40.00	0.00	0.00	0.00	-	-	-	2.0

[1] Ratios were obtained by dividing each MIC by the lowest concentration on the specific antimicrobial tested. The ratio was then turned into a log base 2 for comparison.

[2] Highlighted dash marks indicate breakpoint not on Sensititre® panel.

- = Value greater than the highest number tested on the panel

*Shaded values represent isolates considered resistant for individual drugs based on breakpoints established by the National Antimicrobial Resistance Monitoring System (NARMS).

Table 10. *Salmonella* spp. Mean Minimum Inhibitory Concentrations (MIC) (Log_2 µg/ml) for antimicrobial agents tested

	Market Hogs (n=18)		Market Steers (n=5)	
Antimicrobial Drug	**MIC, µg/ml**	**SE***	**MIC, µg/ml**	**SE**
Cefoxitin	2.00	0.29	1.40	0.24
Azithromycin	2.27	0.10	2.20	0.20
Chloramphenicol	3.22	0.28	2.00	0.31
Tetracycline	5.05	0.39	2.00	0.00
Ceftriaxone	-1.50	0.39	-2.00	0.00
Amoxicillin/clavulanic acid	1.27	0.47	0.00	0.00
Ciprofloxacin	-5.22	0.28	-5.85	0.20
Gentamicin	-1.16	0.34	-2.00	0.00
Nalidixic Acid	2.00	0.28	1.80	0.20
Ceftiofur	0.33	0.25	-0.20	0.37
Sulfisoxazole	7.11	0.45	6.40	0.40
Trimethoprim/sulfamethoxazole	-2.94	0.08	-2.44	0.61
Ampicillin	2.33	0.70	0.00	0.00
Streptomycin	4.88	0.47	3.40	0.24

*SE= Standard Error

isolates and 0.00 $\log_2$ µg/ml for steers. Lastly, market hogs had a mean MIC of 3.22 $\log_2$ µg/ml for Chloramphenicol, while steers had 2.00 $\log_2$ µg/ml.

Overall concern with *Salmonella* contamination is that it can be spread from the environmental surfaces to exhibitors, thereby causing the contamination to spread to other surfaces or people at county fairs and even petting zoos; potentially causing Salmonellosis (Pabilonia et al., 2014; Roug et al., 2013; Keen et al., 2007). If a human were to consume a food product contaminated with antimicrobial resistant bacteria, the outcome could potentially be deadly if the bacterium was virulent enough.

CONCLUSION

Antibiotic resistance is a dynamic issue for both humans and animals. However, little research focuses on where market show animals fit into that dynamic. The aim of this study was to evaluate the prevalence and antimicrobial susceptibility of generic *Escherichia coli* and *Salmonella* spp. in the feces of market show hogs and steers. Results obtained from this study indicate that market show hogs had higher levels of bacterial populations and obtained isolates were consistently more resistant to the tested antimicrobial agents when compared to steers. This study provides additional data to support the need for antimicrobial susceptibility surveillance on both commensal and pathogenic bacteria. This area of research would benefit from a future study that evaluated prior health and diet records as well as collecting additional prevalence and antimicrobial susceptibility data to determine factors that contribute to antimicrobial resistance in market show livestock.

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APPENDIX A

ANGELO STATE UNIVERSITY
College of Graduate Studies & Research
Institutional Animal Care & Use Committee

25 January 2016

Dr. Loree Branham and Ms. Kylee Werland
Department of Agriculture
Angelo State University
San Angelo, TX 76909

Dear Dr. Branham and Ms. Werland:

Your proposed project titled, "Utilizing Rectal Palpation and Fecal Grab Sampling to Obtain Fecal Samples from Multiple Species of Livestock at San Angelo Stock Show and Rodeo" was reviewed by Angelo State University's Institutional Animal Care and Use Committee (IACUC) in accordance with the regulations set forth in the Animal Welfare Act and P.L. 99-158.

This protocol was approved for three years, effective 1February 2016 and it expires three years from this date; however, an annual review and progress report form (www.angelo.edu/content/files/22583-iacuc-annual-review-progressreport) for this project is due on 15 August of each year. If the study will continue beyond three years, you must submit a request for continuation before the current protocol expires.

The protocol number for your approved project is 16-01. Please include this number in the subject line of all future communications with the IACUC regarding the protocol.

Sincerely,

Robert Dowler, Ph.D.
Chair, Institutional Animal Care and Use Committee