ANTIMICROBIAL SUSCEPTIBILITY OF *SALMONELLA* ISOLATES

OBTAINED FROM WEST TEXAS SHEEP:

A STUDY INTO BACTERIAL RESISTANCE

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ABSTRACT

Antibiotic resistance is becoming an increasingly prevalent issue within the health and food production industries. The purpose of this study is to analyze antimicrobial susceptibility in *Salmonella* isolates from small ruminants due to the lack of available literature. *Salmonella* is one bacterial pathogen often found in sheep. Using Sensititre® susceptibility plates, 24 confirmed isolates of *Salmonella* spp. were tested against 15 of the most important antibiotics determined by the National Antimicrobial Resistance Monitoring System. The data obtained was compared against the NARMS determined breakpoints and analyzed using various processes of Statistical Analysis Software (SAS).

Of the 24 isolates tested, 58.33% exhibited resistance to antibiotics, specifically 37.5% were found to be resistant to only one of the antimicrobials tested against, 4.17% were resistant to two antimicrobial drugs, 12.5% of the isolates were resistant to three antimicrobials and 4.17% were resistant to a total of five antimicrobials. The most commonly observed resistance was to Tetracycline at 50%. There were eight antimicrobials that the *Salmonella* isolates showed no significant resistance to, and seven antimicrobials where the minimum inhibitory concentration was found to be higher than the reported breakpoint. Results from this study show that some antibiotic resistance does exist within *Salmonella* obtained from sheep related sources, and prudent use of antibiotics should be advocated to help prevent further spread of resistance.

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INTRODUCTION

Discovering Penicillin in 1928, Alexander Fleming opened the door for the practice of using antibiotics in the animal and human health industries. From that point on the usage of antibiotics only increased over the years. Collective dependency of human and animal medicine on the effectiveness of antibiotics has brought to light the need to minimize the spread of antibiotic resistance in bacteria. A major concern regarding antibiotic resistance is the potential transference of genes from bacteria common to livestock to those that affect humans. The topmost widespread bacteria known to affect both humans and animals include: *Salmonella*, *Campylobacter*, *Enterococcus*, and *Escherichia coli* (McEwen & Fedorka-Cray, 2002).

Antibiotic resistance is a tremendously relevant topic that impacts everyone around the world, whether they are human or animal. Understanding the evolution of bacteria and how they develop resistance to antimicrobials not only impacts food production in the animal industry but facilitates effective treatment of infections in human healthcare as well. As the world's population continues to increase at an exponential rate, it will be up to the agricultural fields to provide enough food to sustain every person. With animal products constituting a large percent of the complete protein sector food supply it is important that antibiotics remain effective in their use as a treatment for infectious diseases.

The United States Department of Agriculture Marketing Service reported 916.5 million pounds of red meat were federally inspected for the week of May 4, 2015. When broken down that amounts to 460.3 million pounds of beef, 451.7 million pounds of pork, and 2.6 million pounds of lamb (United States Department of Agriculture [USDA] Market News,

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2015). The distribution between the three types of red meat is not unexpected since as of 2011 the United States per capita of lamb consumption was only 0.88 pounds. That is however, a considerable drop from the per capita lamb consumption of 1990 which was at 1.6 pounds (Brester, 2012). These statistics show how the demand for lamb has decreased over the years. In other words, Americans consume roughly half a pound of lamb meat a year in comparison to 50 pounds of beef. Despite the diminished popularity of lamb throughout the United States it is still an important food source, especially in certain ethnic markets. According to the American Lamb Board producers can work to bring positive attention back to lamb by targeting certain markets and promoting the consumption of lamb more efficiently (Isaacs, 2012). If this trend continues, having scientific data in regards to antimicrobial resistance from bacteria native to sheep will be extremely beneficial.

SALMONELLA

Salmonella is a gram negative, rod shaped bacilli from the family Enterobacterieaceae. This bacterium was discovered in 1885 by a research laboratory scientist in the Veterinary Division of the United States Department of Agriculture. Shortly after, it was named for Dr. Daniel Salmon, a veterinary pathologist who was the department administrator at the time. Salmonellosis, the illness caused by *Salmonella* bacteria results in diarrhea, fever, abdominal cramps and can lead to acute gastroenteritis, which is inflammation of the stomach and intestines. In most cases recovery is natural and occurs after 4-7 days; however, if the infection becomes invasive and spreads to the urine, blood, bones, joints, brain, or nervous system there can be long-term effects and possibly death if not treated in time. The bacterium can be spread through contaminated food products such as beef, poultry, milk, and eggs or through contact with infected food animals (Centers for Disease Control and Prevention [CDC], 2015b).

According to the Center for Disease Control around 1.2 million illnesses and 450 deaths can be attributed to the bacteria *Salmonella* annually in the United States. Of those *Salmonella* bacteria the National Antimicrobial Resistance Monitoring System has reported that 5% are resistant to five or more antimicrobial agents (CDC, 2015a). In the past, serious *Salmonella* infections were treated with Ampicillin, Trimethoprim-Sulfamethoxazole, or Chloramphenicol. However, nowadays healthcare professionals are more likely to prescribe a quinolone, macrolide, or third generation cephalosporin. Quinolones are a special class of antimicrobial, the most common being ciprofloxacin (CDC, 2015b). NARMS was created in 1966 as a collaborative effort between state and local public health departments, the Center for Disease Control, the United States Food and Drug Administration, and the United States

Department of Agriculture. Their mission is to track the changes in antimicrobial susceptibility in bacteria that affect people, meat products, and production animals (USDA, 2015).

Salmonella in Beef Cattle

Cattle are the primary carriers of *Salmonella* species, and as a result roughly 83% of feedlots administer at least one antimicrobial to their herds as a safety precaution (McEwen & Fedorka-Cray, 2002). This increase in antibiotic usage can also affect the resistance of the natural intestinal flora of the animals. Since beef is one of the top sources of protein in the human diet, this greatly increases the chances of antibiotic resistance transferring to the natural flora found in humans (van den Bogaard & Stobberingh, 2000). As a result, a majority of the research that has been conducted into the susceptibility of *Salmonella* and other bacterial species has been done with cattle.

In 2014, the Animal and Plant Health Inspection Service (APHIS) published findings for the Veterinary Services of the Center for Epidemiology and Animal Health. This study focused on *Salmonella* in U.S. cattle feedlots and the prevalence of antimicrobial resistance. Of the isolates tested, 74.6% were susceptible to all the antimicrobial agents tested against and the antimicrobials that exhibited the most resistance were Tetracycline at 21.4% and Sulfisoxazole at 13.1% (Veterinary Services, 2014).

Salmonella in Humans

According to the 2011 National Antimicrobial Resistance Monitoring Service Executive Report, 85% of *Salmonella* isolates collected from humans exhibited no resistance to the antibiotics they were tested against. This data demonstrates reassuring trends in the effort to restrain antimicrobial resistance (Food and Drug Administration [FDA], 2014a).

However, there are still instances where resistant pathogens are isolated from humans and their origins traced back to farm animals and animal food products. Hospital and veterinary records indicate that animals are treated with antibiotics less frequently than compared to men, women, and children. The fact that the living conditions of animals better favor the development of resistance is a key concern for scientists (Ungemach, Muller-Bahrdt, & Abraham, 2006).

Implications of *Salmonella*

The most significant implication of *Salmonella* infections is the economic burden that the illness places on an individual. A study published in 2004 by the National Centers for Infectious Diseases and the Centers for Disease Control and Prevention determined the average medical expenditure and productivity loss due to *Salmonella.* Medical costs for outpatients included ambulance fee, emergency room services, physician charges, and laboratory tests. Inpatient costs included hospital fees, physician charges, and prescription drug expenses. Productivity loss factors in employment wages and other personal activities. The results found that on average $210 was lost per outpatient, $5,797 per inpatient with gastrointestinal infection, and $16,441 per inpatient with invasive infection. For individuals who chose to treat themselves at home with over the counter medicine the average productivity loss was $53 (Adhikari, Angulo, & Meltzer, 2004).

As an ever evolving country, it can be expected that if this study were conducted in 2014 the economic burden would be much greater. Over the past 10 years both employment rates and most medical costs have increased in response to the higher demands and the development of new technology. Nevertheless, these results provide perspective on the economic implications of *Salmonella* and its developing resistant properties.

LITERATURE REVIEW

The purpose of antibiotics in the health industry is the same for both human and animal. They are indispensable for the treatment and prevention of infectious diseases (Ungemach, Muller-Bahrdt, & Abraham, 2006). However, some producers are using these same antibiotics in the animal industry for the goal of enhancing growth rates and feed efficiency (Wegener, 2003). Irrespective of the intended purpose, some research has shown that excess use of antibiotics can have an impact on the emergence and spread of antibiotic resistant bacteria (van den Bogaard & Stobberingh, 2000). It is an unfortunate side effect that has become increasingly more prevalent as time passes.

Antibiotic Usage

The most common practices of antibiotic usage in livestock include therapeutic, metaphylactic, prophylactic, and growth promotion. Therapeutic administration is the use of antibiotics to control an existing bacterial infection in an individual animal (Schwartz, Kehrenberg, & Walsh, 2001). An examination is conducted by a veterinarian and the symptoms observed are treated with proper dosage and antibacterial agent. If only one animal is exhibiting symptoms but the owner decides to treat the entire herd as a safety measure, this is referred to as metaphylaxis (Schwartz, Kehrenberg, & Walsh, 2001). The only method of administration in this case is through medicated feed and water supplies. When dispensing medication in this way, it is important to take into consideration the possibility that the antimicrobial agent will be incompatible with the feed or insoluble in water and that the animal will receive an uneven mixture or insufficient amount of medicine (Schwartz, Kehrenberg, & Walsh, 2001). In addition, antibiotics are used for the purpose of growth promotion, where they aid in helping the animal digest their food more efficiently and in turn receive the maximum benefit. Some antibiotics are administered for prophylaxis, or solely as a preventive measure. This is most common with specific diseases such as mastitis in dairy cows or during the period of weaning calves (Schwartz, Kehrenberg, & Walsh, 2001).

Antibiotic Resistance

The true origin of antibiotic resistance in bacteria remains a mystery to scientists and researchers. While it is not proven to be a direct result of any of the practices above, connections can be made either way. The existence of bacteria that have a natural resistance to certain antibiotics must also be taken into consideration. With selective pressure these resistant genes can be transferred to other bacteria both horizontally and vertically, even to those that were previously harmless (Schwartz, Kehrenberg, & Walsh, 2001). The main factor that relates to the spread of genes is their location within the bacteria. Resistant genes can be found on both plasmids and chromosomes (Davison, Low, & Woolhouse, 2000). Those genes found on chromosomes are only able to be passed down vertically to the bacterium's daughter cells. Horizontal transference occurs when a bacterium creates a small ring of DNA called a plasmid that can replicate independently of chromosomes and can move between bacteria of the same or different genus/species. Transduction is the main mode of transferring within a species whilst conjugation and mobilization are two ways genes are able to transfer between bacteria of different genus or species (Schwartz, Kehrenberg, & Walsh, 2001). Over time a bacterium can accumulate many different kinds of resistant genes, further mutate ones they already have, or even create new ones. As a result, multi-resistant bacteria have evolved that are capable of protecting themselves from several different varieties of drugs. This encompasses antibiotics that are structurally similar as well as those in different classes that happen to have the same target site (Schwartz, Kehrenberg, & Walsh, 2001).

Implications of Resistance

What scientists fear the most is the transfer of these antibiotic resistant genes to bacteria that can be harmful to humans (van den Bogaard & Stobberingh, 2000). Within the meat industry bacteria can be spread from the carcass of an animal during slaughter or processing and contaminate the food product (Aarestrup & Wegener, 1999). Other methods of dissemination are to the environment through the use of contaminated manure as fertilizer or direct contact from animal to human (Wegener, 2003).

Resistant genes result in reduced effectiveness of the antibiotics used to combat these bacteria (McEwen & Fedorka-Cray, 2002). When looking specifically at the animal industry, an abundance of factors play a role in bacterial resistance: the species of bacteria, the dose of antibiotic used, the duration of treatment, the number of animals exposed, basic husbandry practices, the movement of animals, and potential of environmental spread (McEwen & Fedorka-Cray, 2002). The most debated contributor is the use of antibiotics for the purpose of improved growth rate and feed efficiency (Wegener, 2003).

Production Efficiency

Denmark was the first to act on the unintended consequences of using antibiotics to increase production efficiency. Animal growth results were measured and interpreted; it was found that the benefits were insignificant in comparison to facilitating the spread of resistance (Wegener, 2003). Ranchers and producers voluntarily decided to remove all medicinal feed additives and although the complete disappearance of antibiotic resistance in bacteria has yet to be seen, there has been a considerable decline in resistant infections and resistance in isolated bacteria. The Danish influence had a positive domino effect as other countries such as Sweden were quick to follow suit and ban feed additives (Wegener, 2003).

It is difficult to understand how agents originally intended to protect animal welfare, prevent the spread of infectious diseases, and prevent the possible conveyance to the human population could morph into a means of increasing production efficiency. It is unknown as to when this shift took place but it is speculated to be a result of increased monetary return. There are a few theories discussing how exactly antibiotics improve production. One notion is that the agents work to dampen the effect of disease on growth, possibly by suppressing the bacteria within the host that compete with it for nutrients (McEwen & Fedorka-Cray, 2002). Another hypothesis is that they modulate the metabolic activity of the natural inhabitants of the digestive tract and shift the balance towards better weight gain. Others simply believe that the medications work to enhance the immune system (McEwen & Fedorka-Cray, 2002).

Prevention Techniques

The North American food animal production system has slowly become more intensive over time. It has grown in scale, having developed fewer but much larger farms (van den Bogaard & Stobberingh, 2000). Professionals in the agriculture and healthcare fields have suggested methods to prevent the spread of antibiotic resistant bacteria. As the search for new and improved antibiotics is ongoing, these suggestions are intended to help defend the future effectiveness of the antibiotics being used presently (McEwen & Fedorka-Cray, 2002). Recommendations include reducing the usage, amount and frequency of antibiotics; improving animal husbandry to eradicate the appearance of as many diseases as possible; implementing a veterinary policy; creating surveillance programs; and increasing the number of studies being conducted (van den Bogaard & Stobberingh, 2000).

Animal husbandry practices are carefully managed to ensure the safety of the animal as a top priority. However, as with any organic being, uncontrollable variables could create the possibility of bacterial contamination during harvest (Aarestrup & Wegener, 1999). In the beginning slaughter and processing plants had few protocols. When sanitation and product safety came into the limelight, scientists found a solution by designing the HACCP system (McEwen & Fedorka-Cray, 2002). HACCP stands for Hazard Analysis and Critical Control Points and is used as a preventative measure. Scientific analysis is conducted for the presence of microbiological, physical, and chemical hazards and preventive measures applied throughout the entire process from carcass to food product.

The purpose of surveillance programs is to monitor the occurrence and development of antibiotic resistance as well as the consumption of antibiotics in the hopes of limiting the emergence and distribution (Aarestrup, 1999). Over 13 different countries have implemented some type of monitoring program over the span of years. Denmark has the Danish International Antimicrobial Resistance Monitoring and Research Program and the United States created the National Antimicrobial Resistance Monitoring System in 1996 (McEwen & Fedorka-Cray, 2002). Data is collected and published yearly on the extent and trends of susceptibility. Resistance is identified as soon as possible, and the information is provided to veterinarians and physicians. Overall, their mission is to help extend the lifespan of the drugs already approved to combat disease and infection (van den Bogaard & Stobberingh, 2000).

Monitoring the data is beneficial, but using the information obtained to conduct experiments and research studies can aid in facilitating resolutions to the current problems

(Aarestrup, 1999). The goal is to limit the resistance and still be able to cure infections. In order to obtain this objective, it is important to understand how to properly use and administer antimicrobials, how the resistance is occurring, and the circumstances in which the genes are spreading so rapidly (Aarestrup, 1999). As more knowledge and data is gathered the closer researchers are to the prospect of a solution.

In the meantime, compulsory guidelines to antibiotic usage have been found to have some merit. In 2000, Germany created a set of guidelines that required a veterinarian prescription before any antibiotics could be administered to an animal and then only as a last resort (Ungemach, Muller-Bahrdt, & Abraham, 2006). A veterinarian would possess detailed knowledge of the antibiotics available and the means for a more precise diagnosis. This practice would ensure that a narrow spectrum treatment was chosen that would have a higher margin of safety and the best possible penetration (Ungemach, Muller-Bahrdt, & Abraham, 2006).

Implications of Prevention

The limitations being applied to antibiotics is for the good of the whole. Nevertheless, it is also important to recognize the potential ramifications. Without over the counter access to antibiotics there is an opportunity for an increase in infectious diseases within the animal population (McEwen & Fedorka-Cray, 2002). Even veterinarians would have a limit as to the dosage of antibiotics they could prescribe, which could hinder their ability to treat the animals. A decrease in incentive to discover new drugs might also occur since their use would have the same limitations. From an animal production standpoint, it would be safe to assume that a huge economic impact would occur if stringent guidelines were to be enforced (McEwen & Fedorka-Cray, 2002). Some advocates believe that taking away the ability to use antibiotics as a performance enhancer could reduce stress and permit the animal to grow and produce at their body's natural rate. Providing animals with a safe, sanitary, and comfortable environment is indicative of good management practices and humane treatment. Added stress or contact with infectious bacteria in feed settings has been discovered as a potential driver towards the use of feed additives to improve the animal's production efficiency (McEwen & Fedorka-Cray, 2002). A vital aspect of management includes proper vaccination procedures to maintain a healthy animal that would only require antibiotics in extreme circumstances.

Right now the trend for the prudent use of antibiotics in animal production is the enactment of monitoring systems and strict guidelines (FDA, 2014a). The use of antibiotics is slowly being brought under veterinarian supervision so that only specific authorization is allowed through prescriptions. The hope is to prevent the spread of resistant bacteria and allow antibiotics to revert back to their intended purpose – the treatment, control, and prevention of disease (FDA, 2014b).

PURPOSE

The intent of this study is to explore the subject of antibiotic resistance in *Salmonella* species from small ruminants by testing and analyzing antimicrobial susceptibility. The *Salmonella* isolates used were collected from the feces and hide of sheep located throughout West Texas. Susceptibility and resistance against 15 different antimicrobials of varying concentrations were measured using Sensititre® plates. A minimum inhibitory concentration was determined and compared against the recommended dosage determined by the National Antimicrobial Resistance Monitoring System.

METHODOLOGY

A previous study done by the Angelo State University Agriculture Department collected 205 samples from the feces and hide of West Texas sheep and goats. Of these samples, 43 from sheep tested positive and the presence of a *Salmonella* species confirmed. This research utilized a total of 24 out of the 43 isolates, specifically 12 samples from hide and 12 samples from feces. These isolates were tested against 15 important antibiotics determined by the National Antimicrobial Resistance Monitoring Service using Sensititre® MIC Susceptibility Plates. Antibiotic susceptibility was investigated on the 24 chosen isolates using a Sensititre® Gram Negative NARMS Plate designed by Trek Diagnostic. Antimicrobial breakpoints were obtained from the National Antimicrobial Resistance Monitoring System. Minimum inhibitory concentrations were recorded, converted into log base 2, and analyzed using Statistical Analysis Software.

Each Sensititre® plate consisted of 92 wells containing varying concentrations of 15 specific antibiotics, one well for a positive control, and two wells for a negative control. The concentrations started low and were doubled in each well until the breakpoints met. These antibiotics included: Amoxicillin/Clavulanic Acid, Ampicillin, Azithromycin, Cefoxitin, Ceftiofur, Ceftriaxone, Chloramphenicol, Ciprofloxacin, Gentamicin, Kanamycin, Nalidixic Acid, Streptomycin, Sulfisoxazole, Tetracycline, and Trimethoprim/Sulfamethoxazole.

Materials

Materials used included: *Salmonella* spp. isolates, Tryptic Soy Agar plates, sterilized loops, demineralized water, 0.5 McFarland standard, a vortex mixer, Mueller-Hinton broth, sterilized troughs, a multi-channel pipette, Sensititre® Plates, and an incubator.

Preparation

Previously collected *Salmonella* spp. isolates were incubated on Tryptic Soy Agar plates overnight at 35° C.

Procedure

1) Three to five colonies from the primary agar plates were chosen and emulsified in 4 ml of demineralized water using a sterilized loop.

2) A pipette was used to transfer 10 µL of bacterial suspension into 11 ml of Mueller-Hinton broth.

3) The mixture was vortexed and compared against McFarland standard for visual density.

4) The broth suspension was poured into a sterile trough and using a multi-channel pipette 50 µL was transferred into each well of the Sensititre® plate.

5) The finished plates were covered carefully with an adhesive seal and incubated at 34-36° C for 12-24 hours.

Reading Results

A Sensititre® manual viewer was used to determine bacterial growth. Growth was observed as either turbidity or distinct cells. The lowest concentration of antibiotic that inhibited growth was recorded. If growth was visible in the well with the highest concentration, then an MIC value double the highest concentration was recorded.

RESULTS

The minimum inhibitory concentration was recorded for each antimicrobial and converted into a ratio for comparison. The most significant results found that Amoxicillin/Clavulanic Acid, Ampicillin, Azithromycin, Cefoxitin, Streptomycin, Sulfisoxazole, and Tetracycline all encountered isolates that were resistant above the breakpoint, which is the recommended concentration by the National Antimicrobial Resistance Monitoring System (Table 2). Kanamycin was the only antimicrobial that was found to be 100% effective against *Salmonella* while Ceftriaxone and Trimethoprim/Sulfamethoxazole came close with 95.83%.

Of the 24 *Salmonella* spp. isolates tested, 41.67% were found to be pansusceptible while 58.33% exhibited resistance to at least one antibiotic (Figure 1). Of the total isolates exhibiting resistance, 37.5% were found to be resistant to only one of the antimicrobials tested against, 4.17% were resistant to two antimicrobial drugs, 12.5% of the isolates were resistant to three antimicrobials and 4.17% were resistant to a total of five antimicrobials (Figure 2). The most common resistance observed was to Tetracycline with 50% of the 24 isolates exhibiting resistance to this antimicrobial. Out of the 15 antimicrobials tested, 8 had no recorded resistance from the *Salmonella* isolates. These include Cefoxitin, Nalidixic Acid, Kanamycin, Gentamicin, Trimethoprim/Sulfamethoxazole, Ciprofloxacin, Chloramphenicol, and Ceftriaxone (Figure 3).

The results show that of the 12 fecal isolates, 41.67% were resistant to at least one antimicrobial. More specifically, 25% were resistant to only Tetracycline while 16.67% were resistant to Tetracycline, Streptomycin, and Sulfisoxazole (Table 3). Of the 12 hide isolates, 75% were resistant to at least one antimicrobial. 50% were resistant to Tetracycline, 8.33%

were resistant to Cefoxitin and Amoxicillin/Clavulanic Acid, 8.33% were resistant to Cefoxitin, Amoxicillin/Clavulanic Acid, and Ampicillin, and finally 8.33% were resistant to Tetracycline, Cefoxitin, Amoxicillin/Clavulanic Acid, Ampicillin, and Azithromycin (Table 3). The fecal samples also recorded a 58.3% frequency of susceptibility while the hide samples showed only 25% susceptibility.

Table 1. Antimicrobial drug concentration range and breakpoints.

Antimicrobial Drug	Concentration (μg/ml)	MIC Breakpoint*
Cefoxitin	0.5 - 32	≥ 32
Azithromycin	0.12 - 16	≥ 32
Chloramphenicol	2 - 32	≥ 32
Tetracycline	4 - 32	≥ 16
Ceftriaxone	0.25 - 64	≥ 4
Amoxicillin/Clavulanic Acid	1/0.5 - 32/16	≥ 32/16
Ciprofloxacin	0.015 - 4	≥ 1
Gentamicin	0.25 - 16	≥ 16
Nalidixic Acid	0.5 - 32	≥ 32
Ceftiofur	0.12 - 8	≥ 8
Sulfisoxazole	16 - 256	≥ 512
Trimethoprim/Sulfamethoxazole	0.12/2.38 - 4/76	≥ 4/76
Kanamycin	8 - 64	≥ 64
Ampicillin	1 - 32	≥ 32
Streptomycin	32 - 64	≥ 64

* MIC Breakpoint = Minimum inhibitory concentration breakpoint determined by the National Antimicrobial Resistance Monitoring Service

Table 2. Percentage of isolates (n=24) recovered from feces and hide of West Texas sheep on the basis of MIC ratio.

	MIC Ratio[a]									
Antimicrobial	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Lowest Concentration Tested (µg/ml)**
Amoxicillin/Clavulanic Acid	79.17	4.17	4.17	0.00	0.00	8.33	4.17	—	—	1/0.5
Ampicillin	83.33	4.17	0.00	0.00	4.17	0.00	8.33	—	—	1
Azithromycin	0.00	0.00	0.00	0.00	0.00	12.50	66.67	16.67	4.17	0.12
Cefoxitin	0.00	0.00	37.50	50.00	0.00	0.00	0.00	12.50	—	0.5
Ceftiofur	0.00	0.00	8.33	79.17	12.50	0.00	0.00	—	—	0.12
Ceftriaxone	95.83	4.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25
Chloramphenicol	87.50	12.50	0.00	0.00	0.00	—	—	—	—	2
Ciprofloxacin	83.33	16.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.015
Gentamicin	0.00	8.33	70.83	16.67	4.17	0.00	0.00	—	—	0.25
Kanamycin	100.00	0.00	0.00	0.00	—	—	—	—	—	8
Nalidixic Acid	0.00	54.17	41.67	4.17	0.00	0.00	0.00	—	—	0.5
Streptomycin	91.67	8.33	0.00	—	—	—	—	—	—	32
Sulfisoxazole	0.00	4.17	45.83	41.67	0.00	8.33	—	—	—	16
Tetracycline	50.00	0.00	0.00	4.17	45.83	—	—	—	—	4
Trimethoprim/Sulfamethoxazole	95.83	4.17	0.00	0.00	0.00	0.00	—	—	—	0.12/2.38

[a] Ratios were obtained by dividing each Minimum Inhibitory Concentration by the lowest concentration of the specific antimicrobial tested. The ratio was then transformed to a log base 2 for comparison.

— = Value greater than the highest concentration tested for on the panel.

* Shaded areas represent isolates considered resistant for individual drugs based on breakpoints obtained from the Clinical Laboratory Standards Institute and the National Antimicrobial Resistance Monitoring System.

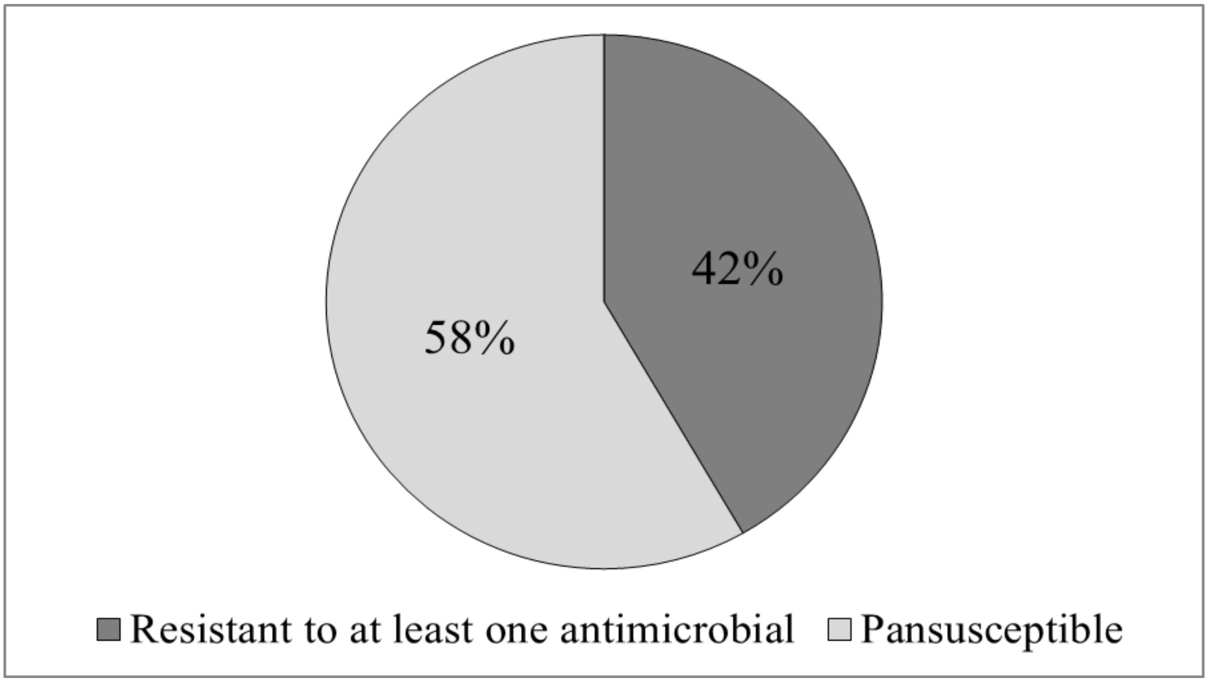

Figure 1. Frequency of antibiotic resistance to at least one antimicrobial in *Salmonella* isolates (n=24) obtained from feces and hide of West Texas sheep.

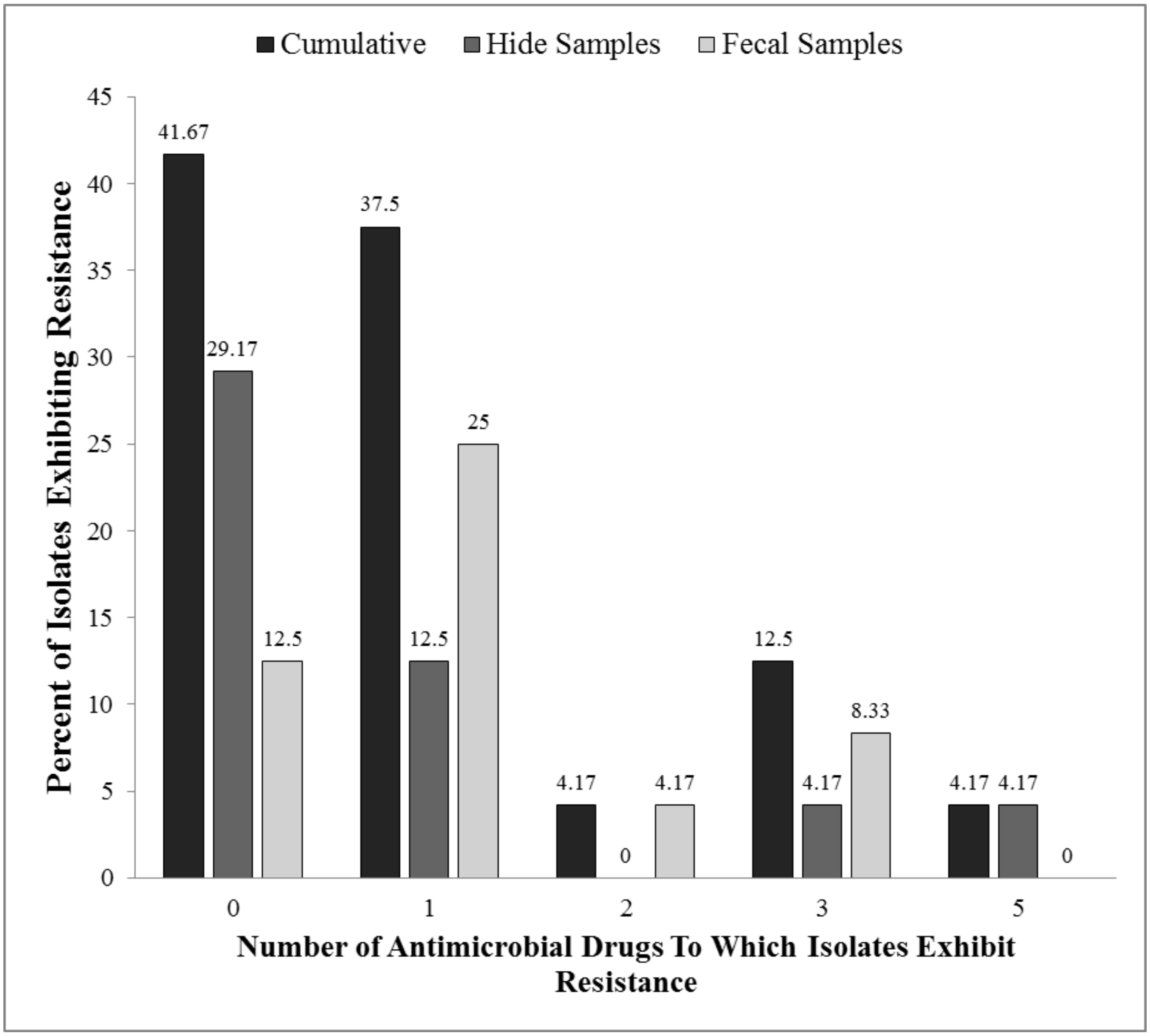

Figure 2. Total percent of *Salmonella* isolates obtained from West Texas sheep based on the number of antimicrobials to which they exhibit resistance (n=24). Total percent of feces and hide *Salmonella* isolates based on the number of antimicrobials to which they exhibit resistance (n=12 Hide + n=12 Fecal).

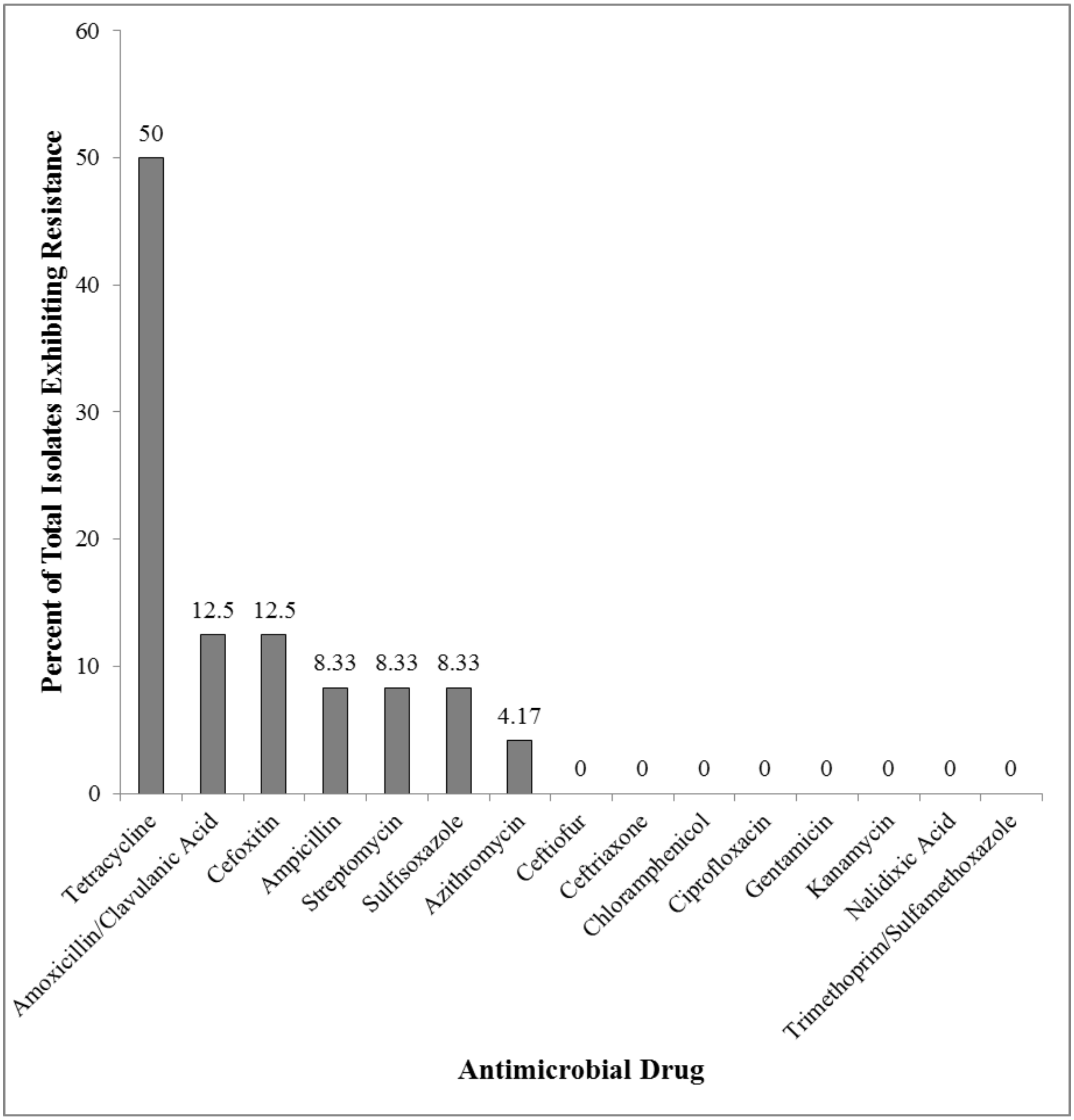

Figure 3. Percent of total *Salmonella* isolates (n=24) collected from feces and hide of West Texas sheep and resistant to tested antimicrobials drugs.

Table 3. Top resistance patterns observed in *Salmonella* isolates obtained from the feces and hide of West Texas sheep.

% Fecal Isolates Resistant (Frequency)	**# of Drugs Resistant**	**Antimicrobials To Which Isolates Were Resistant**
58.33% (n=7)	0	Pansusceptible[a]
25% (n=3)	1	Tetracycline
16.67% (n=2)	3	Tetracycline, Streptomycin, Sulfisoxazole
% Hide Isolates Resistant (Frequency)	# of Drugs Resistant	Antimicrobials To Which Isolates Were Resistant
25% (n=3)	0	Pansusceptible[a]
50% (n=6)	1	Tetracycline
8.33% (n=1)	2	Cefoxitin, Amoxicillin/Clavulanic Acid
8.33% (n=1)	3	Cefoxitin, Amoxicillin/Clavulanic Acid, Ampicillin
8.33% (n=1)	5	Tetracycline, Cefoxitin, Amoxicillin/Clavulanic Acid, Ampicillin, Azithromycin

DISCUSSION

The results of the susceptibility plates expressed a consistent reaction between the antibiotics and the *Salmonella* isolates despite the different collection sources. However, there were some instances where an isolate would show complete resistance to an antimicrobial in one plate and then be completely susceptible to it in another. These variations could be due to gene transference occurring between the species. Further research into genetic identification should be conducted to investigate this possibility. Bacteria have a highly proficient way in which they share their genes. This means that *Salmonella* isolates can inhabit the same environment and yet be more virulent than their neighbors.

Most published studies into antimicrobial susceptibility of *Salmonella* isolates use cattle from feedlot settings as the collection source. When comparing the results obtained from this 2015 study into sheep against a 2014 study using cattle isolates, some commonality was seen. The top antimicrobial exhibiting resistance in both studies was Tetracycline. The next highest in cattle was Sulfisoxazole (Veterinary Services, 2014), while the study into sheep presented a tie between Amoxicillin/Clavulanic Acid and Cefoxitin. Variations in the results could be due to the differing species, environment, or sample size. Further testing with the expressed intention of comparing these variables would be necessary to correlate any of the data obtained.

If this research were to be continued, researchers should repeat the procedure every year at the same time with newly collected Salmonella isolates. Obtaining an MIC ratio over the course of several years would allow for a more in-depth analysis. With this new data a comparison could be made between the MIC ratios to determine if the bacteria are becoming more resistant or more susceptible. As it stands, the results demonstrate that resistant strains of *Salmonella* spp. do exist within the sheep industry and some isolates have exhibited resistance above the recommended concentrations in the health industry. This means that the commonly prescribed dose of the antibiotic would not be sufficient enough to neutralize the bacteria if a human or animal were to become infected.

Limitations

In this study *Salmonella* isolates were obtained from sheep sources in West Texas. As a result several limitations exist that must be taken into account when considering the results. The environmental conditions on the day the sheep samples were collected, as well as the geographical location, can have an impact on the *Salmonella* species isolated for this research and their resistant properties. Another limitation to this research is that a secondary party was responsible for the collection of the samples in the field. The primary researcher did not gain access to these samples until after the *Salmonella* was isolated and incubated on TSA plates. Having firsthand knowledge of all aspects of research allows for more control, organization and elimination of potential errors. Since sheep and goats are both classified as small ruminants, the results determined by this research can be interchanged between both species. To eliminate any inaccuracy resulting from this notion, samples from goats should also undergo antimicrobial susceptibility testing.

The most significant limitation in this research project was the sample size of 24 isolates. This restriction was due to the availability of necessary supplies. Consequently, the number of samples tested is too small to justify using the results obtained as support in making informed management decisions. Despite these limitations, the scarcity of research published in regards to *Salmonella* resistance in small ruminants signifies the results obtained from this project as valuable. This data will be able to provide a solid basis of information that can be built upon through future studies.

Antimicrobial susceptibility in general is an excellent topic for individuals pursuing a career in animal science or food science, as well as human healthcare. This is something that transcends the borders between human and animal and shows how the two worlds are truly connected. As healthcare professionals that others rely on for guidance, it is the responsibility of veterinarians and physicians to understand every aspect of the medications that they prescribe. This includes knowing the complex details of bacterial resistance and its impacts. With antibiotics being regularly prescribed to fight food borne illnesses, those in the food production sector are in a perfect position to help alleviate the pressure by practicing proper antibiotic usage and sanitation. In the end, the struggle to reduce the occurrence and spread of antibiotic resistance will be most effective if a large-scale collaborative effort is maintained.

CONCLUSION

Overall, the greatest amount of susceptibility was recorded in the *Salmonella* samples obtained from fecal matter. The greatest amount of resistance was recorded in the *Salmonella* samples obtained from the hide. The *Salmonella* isolates showed the most resistance to the antibiotic Tetracycline but also showed considerable susceptibility to eight others.

Antibiotic resistance is not a new development; this debate has been a part of the human and animal health industry ever since antibiotics were first discovered. However, concern has continued to grow as the population becomes more involved and aware of the dangers. An important responsibility of healthcare and animal production professionals is to make sure the general population is well informed and educated. As the occurrence of antibiotic resistance increases complications arise in the selection of antimicrobial therapy for serious bacterial infections. Since food animals are the most common source for bacterial species that cause human infections, more research is necessary (Aarestrup, 1999). Of all the food animals, chickens, cattle, and pigs receive the most focus for research. Not much literature has been found in regards to antimicrobial susceptibility in sheep and goats. These species could be just as significant to the spread of resistant bacteria through general contact, fecal and wool contamination, and through dairy products. Antibiotic resistance has now been shown to exist in *Salmonella* isolates obtained from sheep sources. Smaller ruminants must be included as potential risks in the spread of resistance if it is to be contained.

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APPENDIX A: SENSITITRE® PLATE FORMAT

SENSITITRE CUSTOM PLATE FORMAT

Plate Code: CMV2AGNF

	1	2	3	4	5	6	7	8	9	10	11	12
A	FOX 32	AZI 8	CHL 16	AXO 64	AXO 0.25	CIP 2	GEN 16	NAL 16	XNL 2	FIS 32	KAN 64	AMP 2
B	FOX 16	AZI 4	CHL 8	AXO 32	AUG2 32/16	CIP 1	GEN 8	NAL 8	XNL 1	FIS 16	KAN 32	AMP 1
C	FOX 8	AZI 2	CHL 4	AXO 16	AUG2 16/8	CIP 0.5	GEN 4	NAL 4	XNL 0.5	SXT 4/76	KAN 16	STR 64
D	FOX 4	AZI 1	CHL 2	AXO 8	AUG2 8/4	CIP 0.25	GEN 2	NAL 2	XNL 0.25	SXT 2/38	KAN 8	STR 32
E	FOX 2	AZI 0.5	TET 32	AXO 4	AUG2 4/2	CIP 0.12	GEN 1	NAL 1	XNL 0.12	SXT 1/19	AMP 32	NEG
F	FOX 1	AZI 0.25	TET 16	AXO 2	AUG2 2/1	CIP 0.06	GEN 0.5	NAL 0.5	FIS 256	SXT 0.5/9.5	AMP 16	POS
G	FOX 0.5	AZI 0.12	TET 8	AXO 1	AUG2 1/0.5	CIP 0.03	GEN 0.25	XNL 8	FIS 128	SXT 0.25/4.75	AMP 8	POS
H	AZI 16	CHL 32	TET 4	AXO 0.5	CIP 4	CIP 0.015	NAL 32	XNL 4	FIS 64	SXT 0.12/2.38	AMP 4	POS

PLATE TYPE: MIC

ANTIMICROBICS

FOX	Cefoxitin
AZI	Azithromycin
CHL	Chloramphenicol
TET	Tetracycline
AXO	Ceftriaxone
AUG2	Amoxicillin / clavulanic acid 2:1 ratio
CIP	Ciprofloxacin
GEN	Gentamicin
NAL	Nalidixic Acid
XNL	Ceftiofur
FIS	Sulfisoxazole
SXT	Trimethoprim / sulfamethoxazole
KAN	Kanamycin
AMP	Ampicillin
STR	Streptomycin
NEG	Negative Control
POS	Positive Control

VITA

Haleigh Dawn Arent was born in Garland, Texas to Lisa and Jeffrey Arent. At the age of five she moved residence to the state of Nebraska and did not return to Texas until 2009. Haleigh graduated from Angelo State University in May 2015 with a Bachelor of Science in Animal Science. She received Highest University Honors and was the first student to complete the Honors Thesis option for the Department of Agriculture.

Haleigh served as the Student Assistant to the Honors Program from November 2011 until her graduation. Additionally, Haleigh was a member of the Delta Tau Alpha Agriculture Honor Society and the National Honor Society Alpha Chi. She served on the Board of Directors of Concho Valley PAWS for one term through the Honors Program Community Board Initiative. Haleigh was also selected to Who's Who Among Students in American Colleges and Universities. Her individual research *The Cost-Effectiveness of Pre-Adoption Treatment of Heartworms in Rescue Organizations* has been presented at numerous venues. She represented the Angelo State University Honors Program at both the Great Plains Honors Council regional conference as well as at the National Collegiate Honors Council during her senior year.