

PREVALENCE OF ANTIBIOTIC RESISTANT PATHOGENS IN FERAL HOGS OF TEXAS

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ABSTRACT

Fecal samples from feral hogs were collected (n=36) from four locations in Texas including Runnels, Haskell, Crane, and Sutton counties. Samples were analyzed for *Escherichia coli* populations, *Salmonella* spp. and *Listeria* spp. prevalence, and bacterial isolates were collected. Commercial microbroth dilution plates were used to establish antibiotic resistant profiles on the isolates. Non-type specific *E. coli* was found in 91.7% of total samples (n=36) with isolates exhibiting the most common resistance (n=132 isolates) to Sulfisoxazole (46.2%), Tetracycline (2.2%), and Nalidixic acid (2.5%). *Salmonella* spp. was found in 27.7% of total samples with isolates (n=37) showing the most common resistance to Sulfisoxazole (29.7%) and Cefoxitin (2.7%). *Listeria* spp. was found in 22.2% of total samples with isolates (n=29) exhibiting the most common resistance to Lincomycin (89.6%), Daptomycin (68.9%), and Streptomycin (44.8%). This study provides evidence that feral hogs do harbor resistant pathogens that could be foodborne given the right opportunity.

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INTRODUCTION

Feral hogs (*Sus scrofa*) are the most abundant free-ranging introduced ungulate in the United States (Seward et al. 2004). The population is believed to exceed 4 million on a national level (Pimentel et al. 1999), with around 3 million residing in Texas (Muller et al. 2000). Swine first arrived to mainland United States with European settlers as early as the 1500's with the expeditions of Cortez and DeSoto (Towne and Wentworth 1950). Various breeds make up the American feral hog varieties, as well as a more recent hybridization with the Eurasian wild boar (Hutton et al. 2006). By the 1960's, feral hog populations had been known to exist in 20 states (McKnight 1964), and by 2000 the distribution had increased to 39 states (SCWDS 2004).

Feral hogs continue to be a costly, problematic species with the depredation of plant communities and agricultural ecosystems in populated regions (Singer et al. 1982). A group of hogs can uproot and trample large percentages of fields and pasturelands in a single night when feeding, which allows for lost production and decreased harvestability of crops (Tolleson et al. 1995). Feral hogs also destroy bobwhite quail (*Colinus virginianus ridgwayi*) and wild turkey (*Meleagris gallopavo*) nesting sites (Synatzske 1979). Damage to cropland by feral hogs has equaled 800 million dollars annually in the United States, equaling nearly 200 dollars per hog (Pimentel et al. 2002).

In addition to property destruction, feral hogs prey on a variety of livestock, including significant depredation of lambs (*Ovis aries*), goats (*Capra hircus*), and even cattle

(*Bos Taurus*) (Seward et al. 2004). For instance, in the semi–arid regions of Australia, feral hogs can be responsible for up to 32% of predation losses in lambs (Plant et al. 1978).

In addition to physical destruction, feral hogs also pose a threat by harboring diseases transmittable to humans, livestock, pets, and wildlife. Feral hogs are considered highly mobile carriers for at least 30 bacterial and viral diseases (Williams and Barker 2001). Pseudorabies, swine brucellosis, bovine tuberculosis, leptospirosis, and vesicular stomatitis are of large concern when evaluating likely transmission from feral hogs (Williams and Barker 2001). In addition, feral hogs may harbor other bacteria that can cause food-borne illnesses in humans, including *Escherichia coli*, *Salmonella* spp. and *Listeria* spp.

There is growing concern that bacteria responsible for causing food-borne illnesses are becoming resistant to traditional antibiotic treatment. For example, acquired resistance to the antibiotics trimethoprim and the sulphonamides may be encoded on chromosomes due to antibiotic resistant plasmids (Towner 1991). Little research has been done to evaluate antibiotic resistant pathogens in feral hogs, such as non-type specific *Escherichia coli*, *Salmonella* spp. and *Listeria* spp. The purpose of this study was to evaluate non-type specific *Escherichia coli*, *Salmonella* spp. and *Listeria* spp. prevalence in feral hogs of Texas. In addition, the study will attempt to establish antibiotic resistant profiles to evaluate the potential roles of feral hogs as carriers of resistant *Escherichia coli*, *Salmonella* spp. and *Listeria* spp.

OBJECTIVES

1. Evaluate non-type specific *Escherichia coli*, *Salmonella* spp. and *Listeria* spp. population prevalence in feral hogs of Texas.
2. Evaluate antibiotic resistant profiles on *Escherichia coli*, *Salmonella* spp. and *Listeria* spp. in feral hogs.

LITERATURE REVIEW

Damage from Feral Hogs

The distribution of feral hogs in the United States varies based on the quality of the habitat and the history of the local population (Hutton et al. 2006). There are a variety of ways feral hogs have been distributed throughout their habitats, including (1) translocation for hunting purposes, (2) escape from hunting preserves and confinement, (3) avoidance of capture in free-range operations, (4) abandonment by owners, and (5) spread from previously existing feral hog populations (Gipson et al. 1997). In Texas, the most common concern of feral hogs is damage to agricultural crops including hay, small grains, corn, and peanuts (Rollins 1993). In 1988, over \$116,000 of damage was reported to peanut crops alone (Beach 1993). In the early 1990's, livestock depredation by feral swine is listed as a problem for 33% of county agents throughout Texas, with 1,243 sheep and goats documented as lost to feral swine in 1990 alone (Rollins 1993). When a population of feral hogs becomes established in an area, removal of the species becomes increasingly difficult, time consuming, and costly (Hutton et al. 2006). Population explosion and increased suburban encroachments on habitat have increased the incidences of hogs in suburban and urban settings (Agrilife Extension 2008). This increases the chance of human contact with hogs and therefore an increased risk of disease transmission.

Disease Issues Associated with Feral Hogs

Feral hogs have been documented to carry and transmit brucellosis and pseudorabies (Davis 1993). Because of the growing populations of feral hogs in the United States, eradication of swine brucellosis, bovine tuberculosis, and psuedorabies by the USDA

Animal and Plant Health Inspections Service (APHIS) is hindered due to the hog's free-range nature (Witmer et al. 2003). In some places, the feral hog populations can be the most important wildlife host of diseases; therefore they are most able to carry and transmit these diseases to other species through various means (Witmer et al. 2003). There is a concern regarding the possible ability of feral hogs to be a vessel of transmission in an outbreak of a foreign animal disease, such as foot-and-mouth disease or hog cholera, also known as classic swine fever (Witmer et al. 2003). In Louisiana, feral hogs have been documented to contribute *E. coli* into the watershed, and based on the large populations of feral hogs and their affinity to riparian areas, this is most likely not an isolated incident (Kaller et al. 2007).

In the United States, an estimated 73,000 illnesses a year are related to *E. coli* 0157:H7 (Rangel et al. 2005) and 1.4 million cases of Salmonellosis occur annually (White et al. 2001). Listeriosis occurs in as many as 1,860 cases a year such as it did in 1986 (Roberts 1989). Most people can fight off Listeriosis with ease, but pregnant women, infants, and the elderly that are infected with Listeriosis can often die or lose the pregnancy (Roberts 1989). Roberts (1989) also estimated the average cost per Listeriosis case to be at \$135,000 with 510 of the 1,860 (27.4%) cases resulting in mortality.

A nationwide outbreak of *Escherichia coli* 0157:H7 from spinach in California had 205 reported cases and 3 deaths in September of 2006 (Jay et al. 2006). The outbreak was linked to feral hogs and cattle, making the first association of feral hogs and *Escherichia coli* 0157:H7 in the United States with thirteen of 87 (14.9%) of hogs testing positive (Jay et al. 2006).

Antibiotic Resistance and Food Borne Illness

The application of antimicrobial agents to domestic livestock to prevent and treat diseases and support growth is thought by some researchers to be one of the important issues in the materialization of antibiotic resistant bacteria that has the ability to be transferred to humans through contaminated food consumption (White et al. 2001). Strains of bacteria that are resistant to all available antibiotics are uncommon, but resistant strains have been rapidly increasing because of the selective pressure created as susceptible bacteria are killed or inhibited, and resistant bacteria survive and multiply (US Congress, Office of Technology Assessment 1995).

Little, if any, research has been conducted looking for antibiotic resistant *E. coli*, *Salmonella* spp. and *Listeria* spp. in feral hogs. However, some research has shown that the bacteria *Clostridium* spp., that was isolated from the cecal contents of feral hogs, showed high levels of resistance to certain antibiotics of veterinary and human health importance (Ramlachan et al. 2007). More research is needed to investigate resistance levels of common food borne bacteria harbored within feral hogs.

MATERIAL AND METHODS

Four locations in west Texas were used to collect fecal samples from feral hogs. Property in Runnels, Haskell, Crane, and Sutton counties were utilized to represent the west Texas region, and the respective amounts of 12, 7, 13, and 4 hogs were harvested from these counties. These locations are involved in feral hog control efforts, allowing for viable harvesting to obtain the samples. Sites were selected because they contained existing hog control efforts.

Enumeration and Isolation

Fecal samples of 50 or more grams were obtained from individual animals. The number of animals harvested from each property varied. Samples were collected via direct rectal grab or through aseptic extraction from the rectum immediately after harvesting. Immediately following collection, they were placed in labeled individual sterile collection containers and temporarily stored in coolers. When all samples from the location were collected, samples were transported at 4°C to the Angelo State University Food Microbiology Laboratory within 48 hours of initial collection.

To isolate *E. coli*, all samples were plated and enumerated on MacConkey agar using the spread plate method and incubated at 37°C for 24 h (Zimbro et al. 2009). Following enumeration, the four most isolated and distinct colonies were re-isolated onto fresh MacConkey agar and incubated for 24 h at 37°C. Standard *E. coli* colonies are pink to red in color which makes differentiation possible (Zimbro et al. 2009).

To isolate the *Salmonella* spp., 1g of fecal matter was placed in test tubes with 9mL of Tetrathionate broth and vortexed to ensure thorough distribution of the fecal contents. The tubes were then incubated at 37°C for 24 h for selective enrichment. Selective plating then took place for isolation of *Salmonella* spp. by plating on XLT4 agar and incubating at 37°C for 48 h. Typical colonies were red or yellow in color with black centers which allowed for differentiation (Zimbro et al. 2009).

To isolate the *Listeria* spp., 1g of the fecal contents were added to 9mL of *Listeria* enrichment broth and vortexed to ensure thorough distribution of the fecal contents. The test tubes were incubated for 48h at 30°C. Selective plating then took place for isolation of *Listeria* spp. by plating to Modified Oxford agar and incubating for 48h at 30°C. Typical colonies were clear with black staining on the surrounding agar which allowed for differentiation (Zimbro et al. 2009).

Upon completion of the isolation, the colonies were individually transferred into 9 mL Brain-Heart Infusion (BHI) broth test tubes and incubated at 37°C for 24-48 h. The test tubes were then vortexed to redistribute the bacteria culture. A 1 mL fraction of each isolation culture, as well as 1 mL of a glycerol solution (80% glycerol, 20% sterile deionized water), was placed into a sterile cryogenic tube and labeled with a distinctive code and stored in a -80°C freezer for later analysis.

Antibiotic Susceptibility

The frozen isolates were taken from the freezer and thawed at room temperature for 10 minutes. The culture was streaked onto a Tryptic Soy Agar petri-plate and incubated

at 37°C for 24 h. The most viable three to five colonies were isolated and transferred to 5 mL sterile water test tubes and adjusted to a 0.5 McFarland standard for turbidity. Ten microliters of the solution was transferred to 10 mL Mueller-Hinton broth. For each individual isolate, minimum inhibitory concentrations (MIC) of 15 antimicrobials of both human and veterinary significance were determined with the application of a commercial microbroth dilution 96-well, gram-negative and gram-positive, Sensititre® plate (TREK Diagnostic Systems Cleveland, Ohio). The wells were inoculated with 50 µL per well and incubated at 36°C for 18-24 h.

Generic *E. coli* and *Salmonella* spp. were tested on the gram negative plate include Amikacin, Ampicillin, Amoxicillin/Clavulanic Acid, Ceftriaxone, Chloramphenicol, Ciprofloxacin, Trimethoprim/Sulfamethoxazole, Cefoxitin, Gentamicin, Kanamycin, Nalidixic Acid, Sulfisoxazole, Streptomycin, Tetracycline, and Ceftiofur (Table 1). The *Listeria* spp. isolates were tested on the gram positive plate, which included Chloramphenicol, Ciprofloxacin, Daptomycin, Erythromycin, Gentamicin, Kanamycin, Lincomycin, Linezolid, Nitrofurantoin, Penicillin, Quinupristin/Dalfopristin, Streptomycin, Tetracycline, Tigecycline, Tylosin tartrate, and Vancomycin (Table 2). Minimum inhibitory concentrations were determined for each microbial, and isolates were classified as resistant or susceptible using predetermined breakpoints (CDC 2007, CLSI 2006).

Descriptive statistics were generated using various procedures of SAS (SAS Institute, Cary NC, Version 9.1.3). Descriptive statistics include the frequencies of: resistance levels to individual antibiotics, resistance to varying numbers of antibiotics, specific antibiotic

Antimicrobial Drug	Concentration ($\mu\text{g/mL}$)	MIC Breakpoint ($\mu\text{g/mL}$)*
Amikacin	0.5-64	≥ 64
Ampicillin	1-32	≥ 32
Amoxicillin/Clavulanic Acid	1/0.5-32/16	$\geq 32/16$
Ceftriaxone	0.25-64	≥ 64
Chloramphenicol	2-32	≥ 32
Ciprofloxacin	0.015-4	≥ 4
Trimethoprim/Sulfamethoxazole	0.12/2.38-4/76	$\geq 4/76$
Cefoxitin	0.5-32	≥ 32
Gentamicin	0.25-16	≥ 16
Kanamycin	8-64	≥ 64
Nalidixic Acid	0.5-32	≥ 32
Sulfisoxazole	16-256	≥ 256
Streptomycin	32-64	≥ 64
Tetracycline	4-32	≥ 32
Ceftiofur	0.12-8	≥ 8

Table 1. Gram-Negative Antimicrobial drug concentration range and breakpoints.

*MIC Breakpoint = Minimum Inhibitory Concentration Breakpoint, obtained from the Clinical Laboratory Standards Institute and the National Antimicrobial Resistance Monitoring System.

Antimicrobial Drug	Concentration (µg/mL)	MIC Breakpoint (µg/mL)*
Chloramphenicol	2-32	≥32
Ciprofloxacin	0.12-4	≥4
Daptomycin	0.25-16	≥16
Erythromycin	0.25-8	≥8
Gentamicin	128-1024	≥512
Kanamycin	128-1024	≥1024
Lincomycin	1-8	≥16
Linezolid	0.5-8	≥8
Nitrofurantoin	2-64	≥64
Penicillin	0.25-16	≥16
Quinupristin/Dalfopristin	0.5-32	≥4
Streptomycin	512-2048	≥1024
Tetracycline	1-32	≥16
Tigecycline	0.015-0.5	≥0.5
Tylosin tartrate	0.25-32	≥32
Vancomycin	0.25-32	≥32

Table 2. Gram-Positive Antimicrobial drug concentration range and breakpoints.

*MIC Breakpoint = Minimum Inhibitory Concentration Breakpoint, obtained from the Clinical Laboratory Standards Institute and the National Antimicrobial Resistance Monitoring System.

patterns, as well as *Salmonella* and *Listeria* spp. incidence rates. Significant differences between *E. coli* populations were evaluated using a predetermined α of ≤ 0.05 using the MIXED procedure of SAS.

RESULTS

Generic *E. coli*

One hundred thirty-two generic *E. coli* isolates were analyzed to determine the levels of resistance to 15 antimicrobials of importance to human and veterinary health. Of these, 51.5% (68 of 132) were susceptible to all antibiotics in the study, considered pansusceptible, with 47.8% (61 of 132) being resistant to at least one antibiotic and 2.3% (3 of 132) being resistant to two antibiotics (Fig 1). Sulfisoxazole exhibited the largest amount of resistant isolates with 43.9% (58 of 132) of the isolates showing growth at or past the breakpoint (Table 3, Table 4). The numbers of isolates resistant were much lower with Tetracycline, Nalidixic acid, and Ceftiofur, which were resisted by 2.3%, 1.5%, and 0.8% of the isolates, respectively (Fig 2). Only three isolates total showed resistance to two or more antimicrobials (Fig 1). Two of the isolates (1.5%) were resistant to Sulfisoxazole and Nalidixic acid and one isolate (0.8%) was resistant to Sulfisoxazole and Tetracycline (Table 3).

The highest presence of isolates resistant to any antibiotic was in Runnels County, with 65.9% (29 of 44) of the isolates resistant to at least one antimicrobial, followed by Haskell, Sutton, and Crane counties at 45.8%, 41.7%, and 36.5%, respectively. The number of samples testing positive by county can be found in Table 5. Runnels County also accounted for all three of the isolates that exhibited resistance to two or more antimicrobials, as well as having a representation in five out of the six resistance patterns that all counties exhibited. Runnels County also housed the only *E. coli* isolates

Table 3. Most frequent antibiotic drug resistance patterns of Generic *E. coli* isolates obtained from fecal samples from feral hog in West Texas (n=132).

Antimicrobials to Which Isolates Were Resistant	# of Drugs Resistant	% Isolates (Frequency)
Pansusceptible*	0	51.52 (68)
Sulfisoxazole	1	43.94 (58)
Tetracycline	1	1.52 (2)
Sulfisoxazole, Nalidixic Acid	2	1.52 (2)
Ceftiofur	1	.76 (1)
Sulfisoxazole, Tetracycline	2	.76 (1)

*Pansusceptible=susceptible to all antibiotic drugs tested

Table 4. Percentage of Generic *E. coli* isolates (n=132) taken from feral hog feces on the basis of minimum inhibitory concentration ratio.

Antimicrobial	Generic <i>E. coli</i> MIC ratio										Lowest Concentration tested µg/ml
	0	1	2	3	4	5	6	7	8	9	
Amikacin	—	1.52	48.48	46.97	3.03	—	—	—	—	—	0.5
Ampicillin	15.15	83.33	1.52	—	—	—	—	—	—	—	1
Amoxicillin/Clavulanic Acid	2.27	61.36	35.61	0.76	—	—	—	—	—	—	1/5
Ceftriaxone	96.97	—	1.52	0.76	—	—	0.76	—	—	—	0.25
Chloramphenicol	0.76	89.39	9.85	—	—	—	—	—	—	—	2
Ciprofloxacin	81.82	13.64	1.52	1.52	1.52	—	—	—	—	—	0.015
Trimethoprim/Sulfamethoxazole	98.48	0.76	0.76	—	—	—	—	—	—	—	0.12/2.38
Cefoxitin	—	—	12.12	73.48	13.64	0.76	—	—	—	—	0.5
Gentamicin	—	50.76	46.97	2.27	—	—	—	—	—	—	0.5
Kanamycin	99.24	0.76	—	—	—	—	—	—	—	—	8
Nalidixic Acid	—	44.70	52.27	0.76	—	0.76	1.52	—	—	—	0.5
Sulfisoxazole	6.06	18.18	15.91	13.64	46.21	—	—	—	—	—	16
Streptomycin	100.00	—	—	—	—	—	—	—	—	—	32
Tetracycline	97.73	—	—	1.52	0.76	—	—	—	—	—	4
Ceftiofur	0.76	19.70	74.24	3.79	0.76	—	0.76	—	—	—	0.12

¹Ratios were obtained by dividing each minimum inhibitory concentration by the lowest concentration of the specific antimicrobial tested. The ratio was then turned into a log base 2 for comparison.

—=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 Clinical Laboratory Institute and the National Antimicrobial Resistance Monitoring System.

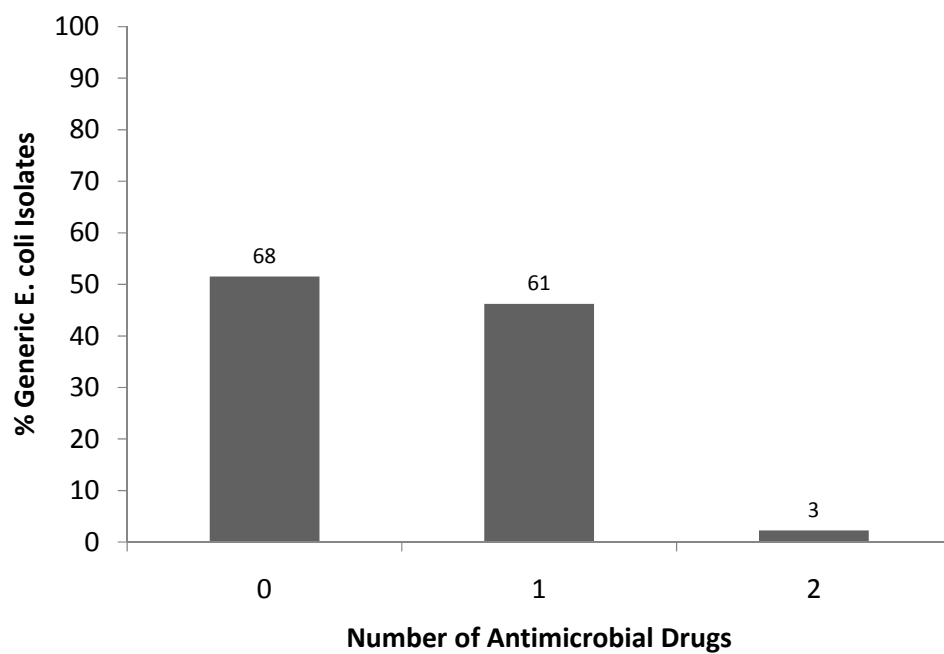
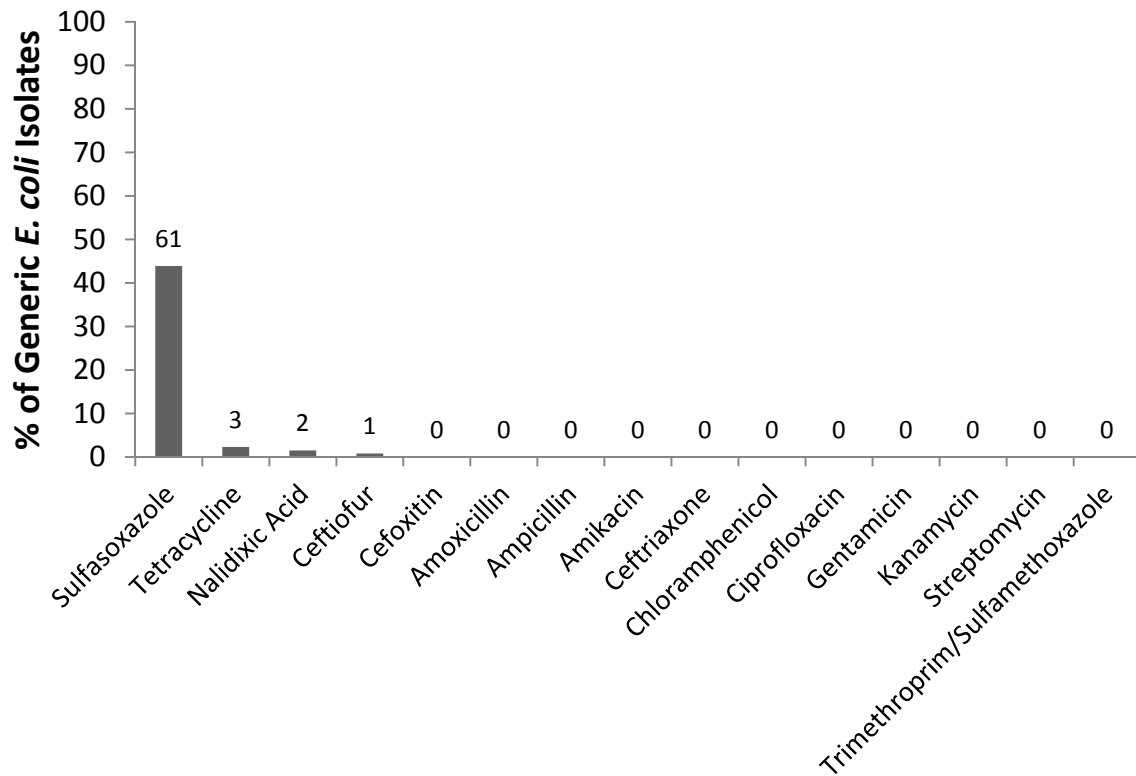


Figure 1. Percent of Generic *E. coli* Isolates (n=132) from Feral Hog Fecal Samples Exhibiting Resistance.

*Frequency of resistant isolates located above bars.



Antimicrobial Drugs

Figure 2. Percent of Generic *E. coli* Isolates (n=132) from Feral Hog Fecal Samples Resistant to Antimicrobial Drugs.

*Frequency of resistant isolates located above bars.

Table 5. Prevalence of Generic *E. coli*, *Listeria* spp., and *Salmonella* spp. in Feral Hog Fecal Samples by County.

Location (County)	# of animals Sampled	<i>E. coli</i> prevalence % pos. (frequency)	<i>Listeria</i> spp. prevalence % pos. (frequency)	<i>Salmonella</i> spp. prevalence % pos. (frequency)
Runnels	12	91.7 (11)	33.3 (4)	41.7 (5)
Haskell	7	85.7 (6)	57.1 (4)	0 (0)
Crane	13	100 (13)	0 (0)	30.8 (4)
Sutton	4	75 (3)	0 (0)	25 (1)
Total	36	91.7 (33)	22.2 (8)	27.7 (10)

Frequency of prevalence located in parenthesis

that were resistant to Tetracycline and Nalidixic acid, with Crane County accommodating the only Ceftiofur resistant isolate.

***Salmonella* spp.**

Thirty-seven *Salmonella* spp. isolates were analyzed to determine levels of resistance to 15 antimicrobials of importance to human and veterinarian health. Of these, 64.9% (24 of 37) were susceptible to all antibiotics in the study, 32.4% (12 of 37) were resistant to one antibiotic, and 2.70% (1 of 37) were susceptible to three antibiotics (Fig 3). The most common resistance was to Sulfisoxazole, Cefoxitin, Amoxicillin/Clavulanic acid, and Ampicillin with the respective values of 29.7% , 5.4%, 2.7% and 2.7% of isolates showing resistance to these specific antimicrobials (Fig 4). Three distinct patterns of resistance were exhibited (Table 7) with one isolate showing resistance to three antibiotics. The complete *Salmonella* spp. resistance profile can be seen in Table 6.

Runnels, Sutton and Crane counties all had samples which tested positive for *Salmonella* spp. (Table 5). Sutton County had only one sample which tested positive for *Salmonella* spp. However, three of the four isolates collected from this sample showed resistance to Sulfisoxazole. Runnels County had six resistant isolates with Crane County having only four, but Crane County did have the one isolate that was resistant to Ampicillin, Amoxicillin, and Cefoxitin.

***Listeria* spp.**

Twenty-nine *Listeria* spp. isolates were analyzed to determine levels of resistance to sixteen antimicrobials of importance to human and veterinary health. Of these isolates,

Table 6. Percentage of *Salmonella* spp. isolates (n=37) taken from feral hog feces on the basis of minimum inhibitory concentration ratio.

Antimicrobial	<i>Salmonella</i> spp. MIC ¹ ratio										Lowest concentration tested µg/ml
	0	1	2	3	4	5	6	7	8	9	
Amikacin	2.70	78.38	18.92	—	—	—	—	—	—	—	0.5
Ampicillin	91.89	2.70	—	—	2.70	—	2.70	—	—	—	1
Amoxicillin/Clavulanic Acid	89.19	5.41	—	—	2.70	2.70	—	—	—	—	1/5
Ceftriaxone	94.59	2.70	2.70	—	—	—	—	—	—	—	0.25
Chloramphenicol	—	94.59	5.41	—	—	—	—	—	—	—	2
Ciprofloxacin	97.30	2.70	—	—	—	—	—	—	—	—	0.015
Trimethoprim/Sulfamethoxazole	100.00	—	—	—	—	—	—	—	—	—	0.12/2.38
Cefoxitin	—	2.70	43.24	43.24	5.41	—	—	5.41	—	—	0.5
Gentamicin	2.70	86.49	8.11	2.70	—	—	—	—	—	—	0.5
Kanamycin	100.00	—	—	—	—	—	—	—	—	—	8
Nalidixic Acid	—	40.54	59.46	—	—	—	—	—	—	—	0.5
Sulfisoxazole	5.41	8.11	18.92	37.84	29.73	—	—	—	—	—	16
Streptomycin	100.00	—	—	—	—	—	—	—	—	—	32
Tetracycline	100.00	—	—	—	—	—	—	—	—	—	4
Ceftiofur	—	—	16.22	81.08	2.70	—	—	—	—	—	0.12

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

—=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 Clinical Laboratory Institute and the National Antimicrobial Resistance Monitoring System.

Table 7. Most frequent multiple antibiotic drug resistance patterns of *Salmonella* spp. isolates obtained from feral hog feces.

Antimicrobials to Which Isolates Were Resistant	# of Drugs Resistant	% Isolates (Frequency)
Pansusceptible*	0	64.86 (24)
Sulfisoxazole	1	29.73 (11)
Cefoxitin	1	2.7 (1)
Ampicillin, Amoxicillin/Clavulanic acid, Cefoxitin	3	2.7 (1)

*Pansusceptible=susceptible to all antibiotic drugs tested

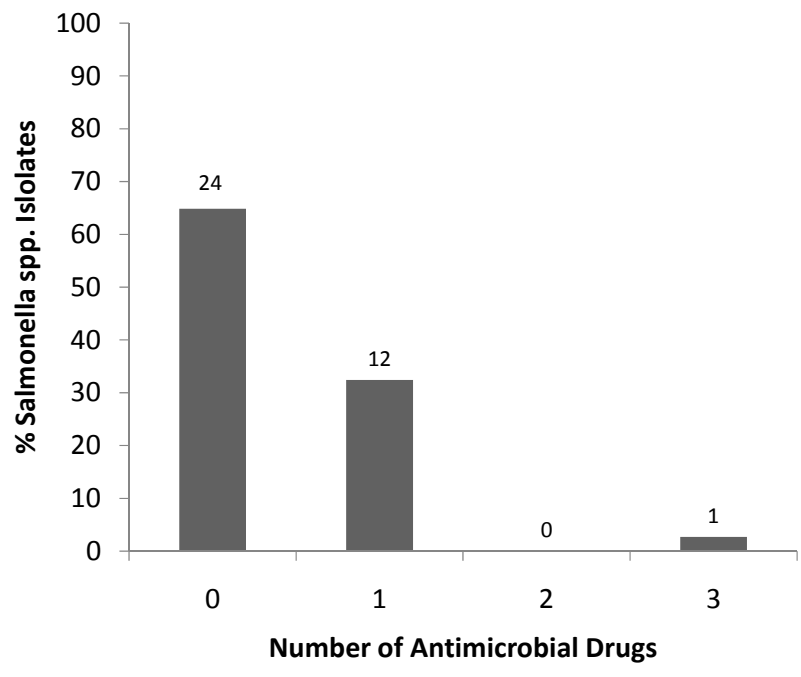
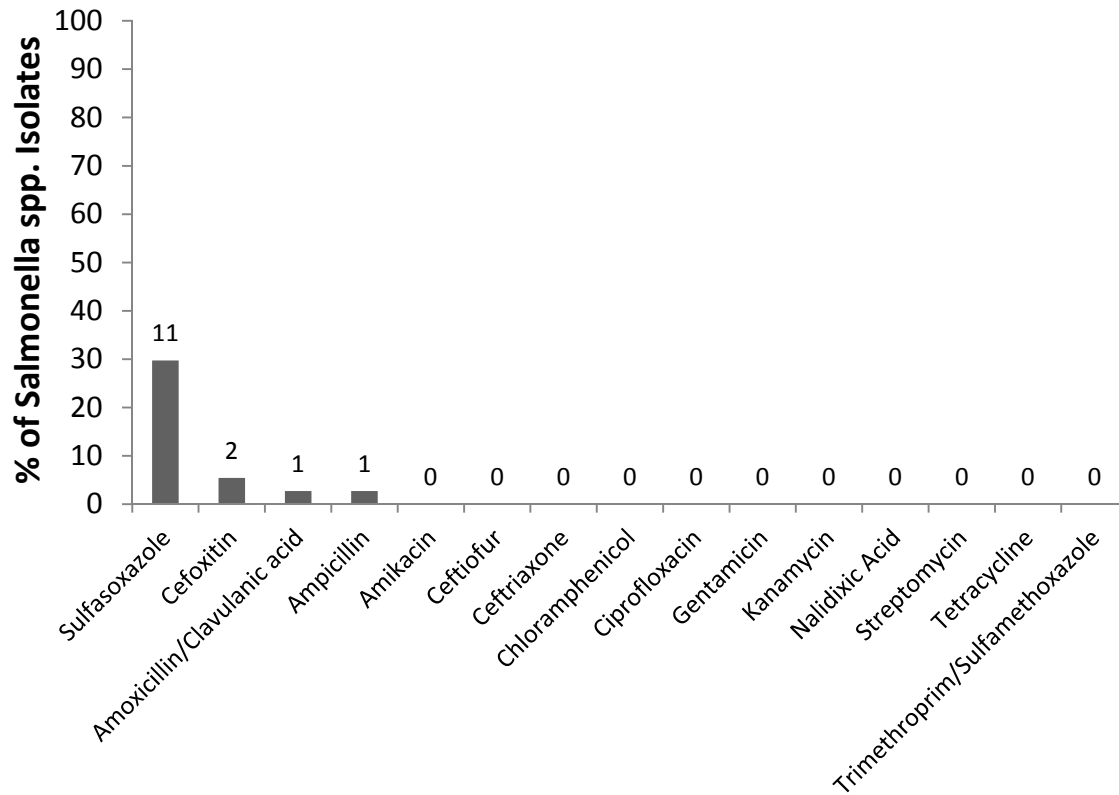


Figure 3. Percent of *Salmonella* spp. Isolates (n=37) from Feral Hog Fecal Samples Exhibiting Resistance.
Frequency of resistant isolates located above bars.



Antimicrobial Drugs

Figure 4. Percent of *Salmonella* spp. Isolates (n=37) from Feral Hog Fecal Samples Resistance to Antimicrobial Drugs.
Frequency of resistant isolates located above bars.

10.3% (4 of 29) (Fig 6) were pansusceptible and 89.7% (26 of 29) exhibited resistance to at least one microbial (Table 8). The most common resistance was to Lincomycin, followed by Daptomycin and Streptomycin at the respective amounts of 89.7% (26 of 29), 69.0% (20 of 29), and 44.8% (13 of 29) of the isolates showing resistance (Fig 5). Fourteen distinct patterns of antibiotic resistance were exhibited by the isolates (Table 9) with one isolate exhibiting resistance to as many as nine antibiotics (Fig 6).

Haskell and Runnels counties were the only areas with samples that tested positive for any *Listeria* spp. with 57.1% (4 of 7) and 36.4% (4 of 11) of the hogs in each respective county exhibiting *Listeria* spp (Table 5). Runnels County isolates were resistant to antibiotics that Haskell County isolates were susceptible to such as Chloramphenicol, Tylosin tartrate, Linezolid, Penicillin, Erythromycin, and Vancomycin. Three Runnels County isolates exhibited a pattern of resistance to eight commercial antibiotics and one isolate was resistant to nine antibiotics. Although the Haskell County's *Listeria* spp. isolates showed resistance to a fewer number of antibiotics, the county had a higher percentage of hogs that tested positive for *Listeria* spp. (57.1%).

Table 8. Percentage of *Listeria* spp. isolates (n=29) taken from feral hog feces on the basis of minimum inhibitory concentration ratio.

Antimicrobial	<i>Listeria</i> spp. MIC ¹ ratio										Lowest concentration tested µg/ml
	0	1	2	3	4	5	6	7	8	9	
Chloramphenicol	13.79	6.90	37.93	34.48	6.90	—	—	—	—	—	2
Ciprofloxacin	—	6.90	24.14	58.62	10.34	—	—	—	—	—	0.12
Daptomycin	—	3.45	13.79	3.45	—	10.34	34.48	34.48	—	—	0.25
Erythromycin	13.79	3.45	17.24	41.38	3.45	—	20.69	—	—	—	0.25
Gentamicin	100.00	—	—	—	—	—	—	—	—	—	128
Kanamycin	100.00	—	—	—	—	—	—	—	—	—	128
Lincomycin	—	—	—	10.34	89.66	—	—	—	—	—	1
Linezolid	6.90	17.24	58.62	3.45	—	13.79	—	—	—	—	0.5
Nitrofurantoin	—	3.45	20.69	51.72	10.34	3.45	10.34	—	—	—	2
Penicillin	13.79	10.34	20.69	24.14	13.79	3.45	10.34	3.45	—	—	0.25
Quinupristin/Dalfopristin	17.24	17.24	48.28	3.45	—	—	13.79	—	—	—	0.5
Streptomycin	55.17	—	44.83	—	—	—	—	—	—	—	512
Tetracycline	75.86	3.45	—	—	13.79	—	6.90	—	—	—	1
Tigecycline	17.24	13.79	55.17	13.79	—	—	—	—	—	—	0.015
Tylosin tartrate	3.45	3.45	13.79	6.90	17.24	31.03	6.90	—	17.24	—	0.25
Vancomycin	10.34	37.93	27.59	6.90	3.45	3.45	10.34	—	—	—	0.25

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

—=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 Clinical Laboratory Institute and the National Antimicrobial Resistance Monitoring System. When breakpoints were not available, the highest dilution available was used to classify as resistant.

Table 9. Most frequent multiple antibiotic drug resistance patterns of *Listeria* spp. isolates obtained from feral hog feces.

Antimicrobials to Which Isolates Were Resistant	# of Drugs Resistant	% Isolates Resistant
Daptomycin, Streptomycin, Lincomycin	3	27.59 (n=8)
Pansusceptible*	0	10.34 (n=3)
Daptomycin, Tylosin Tartrate, Quinupristin/Dalfopristin, Linezolid, Penicillin, Erythromycin, Vancomycin, Lincomycin	8	10.34 (n=3)
Daptomycin, Lincomycin	2	6.9 (n=2)
Tetracycline, Daptomycin, Lincomycin	3	6.9 (n=2)
Nitrofurantoin, Lincomycin	2	6.9 (n=2)
Tetracycline, Chloramphenicol, Daptomycin, Lincomycin	4	3.45 (n=1)
Streptomycin, Lincomycin	2	3.45 (n=1)
Tetracycline, Daptomycin, Streptomycin, Lincomycin	4	3.45 (n=1)
Daptomycin, Streptomycin, Quinupristin/Dalfopristin, Lincomycin	4	3.45 (n=1)
Streptomycin, Nitrofurantoin, Lincomycin	3	3.45 (n=1)
Daptomycin, Streptomycin, Nitrofruantoin, Lincomycin	4	3.45 (n=1)
Erythromycin, Lincomycin	2	3.45 (n=1)
Tetracycline, Chloramphenicol, Tylosin Tartrate, Erythromycin, Lincomycin	5	3.45 (n=1)
Tetracycline, Daptomycin, Tylosin Tartrate, Quinupristin/Dalfopristin, Linezolid, Penicillin, Erythromycin, Vancomycin, Lincomycin	9	3.45 (n=1)

*Pansusceptible=susceptible to all antibiotic drugs tested

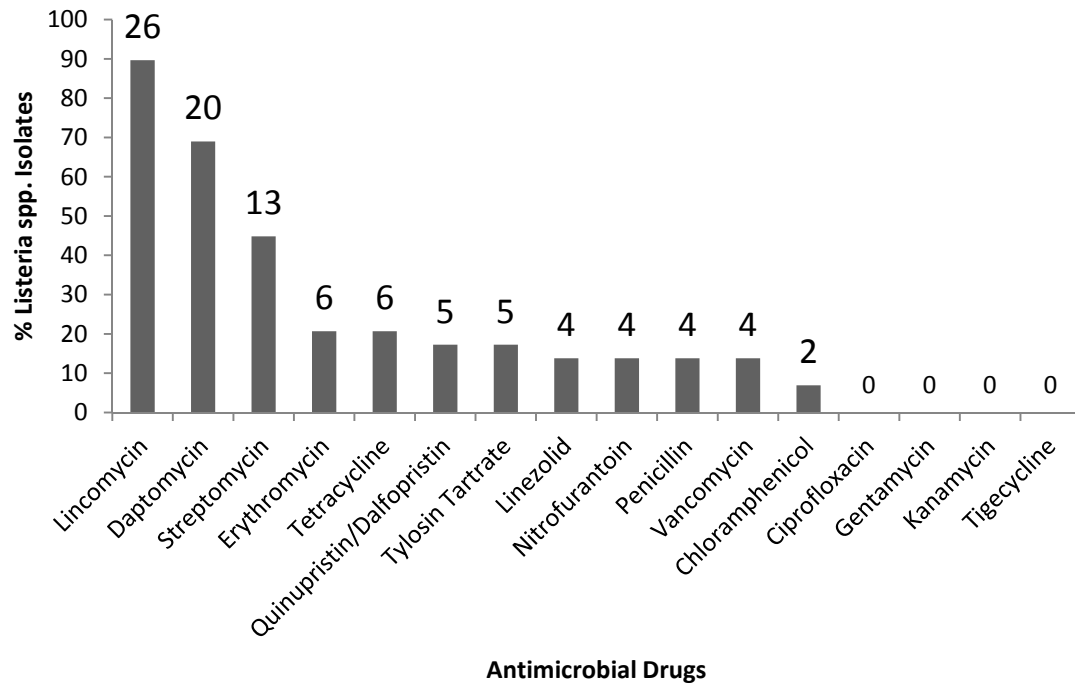


Figure 5. Percent of *Listeria* spp. Isolates from Feral Hog Fecal Samples (n=29) Exhibiting Resistance.
Frequency of resistant isolates located above bars.

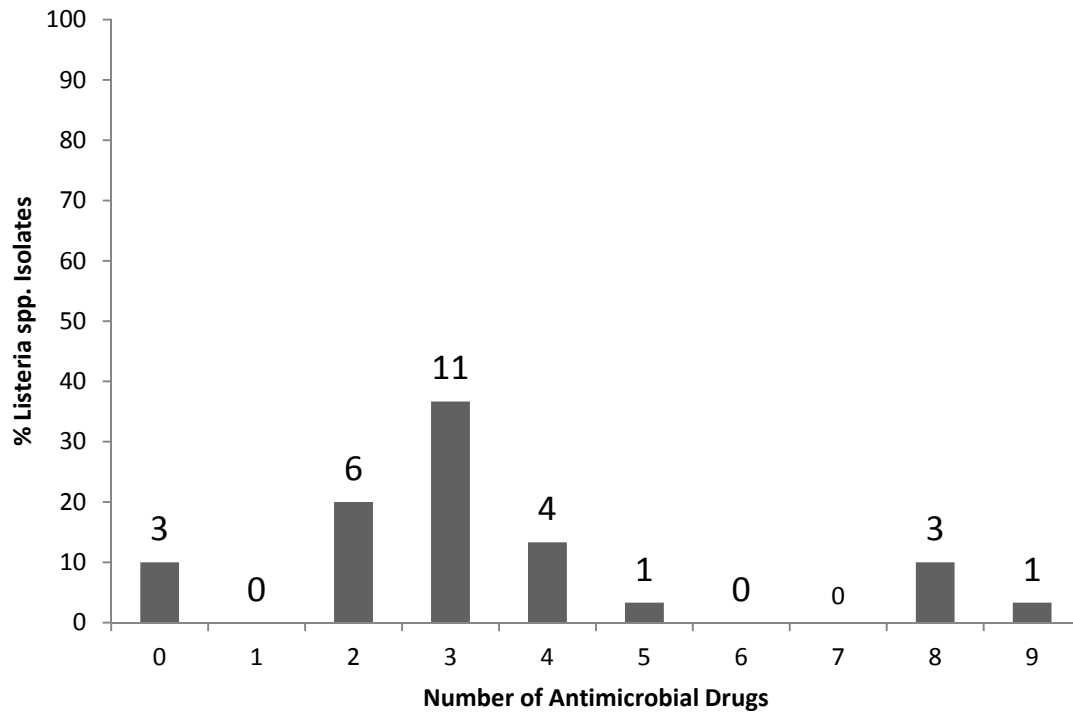


Figure 6. Percent of *Listeria* spp. Isolates (n=29) from Feral Hog Fecal Samples Exhibiting Resistance.
Frequency of resistance located above bars.

DISCUSSION

At least a portion of all *E. coli*, *Salmonella* spp., and *Listeria* spp. isolated exhibited some level of resistance to traditional antibiotics. The *Listeria* spp. resistance encountered was much more diverse than the generic *E. coli* or *Salmonella* spp. in frequency and the patterns of resistance. The resistance patterns of two *Listeria* spp. isolates which resisted the most antibiotics (8 and 9 antibiotics resisted, 13.3% or n=4) both exhibited Penicillin resistance, which is of major importance when the treatment of choice is usually Penicillin in combination with an aminoglycoside such as Gentamicin (Conter et al. 2009).

Gentamicin, however, was not resisted by *Listeria* spp. in this study, showing that it is still a viable treatment for listeriosis, but only if it is administered without the Penicillin.

Kanamycin has been combined with Penicillin as well to have a more complete and earlier killing of *Listeria* spp. than treating with a single antibiotic (Gordon et al. 1972). There was no *Listeria* spp. resistance to Kanamycin in this study.

Tetracycline and Erythromycin are both alternative treatments for individuals that are allergic to Penicillin, and the isolate that was resistant to nine antibiotics resisted all three of the possible treatments of Penicillin, Erythromycin, and Tetracycline (Roberts et al. 1996). This could prove to be extremely dangerous if an immunocompromised individual (elderly, neonatal, AIDS carriers) contracted such a pathogen, as treatment is severely hindered (Farber and Peterkin 1991). Overall, Tetracycline and Erythromycin were resisted simultaneously in two isolates in *Listeria* spp. (6.7%). The resistance to Tetracycline and

Erythromycin has likely occurred from acquiring plasmids from the genera *Enterococcus* and *Streptococcus* (Poyart-Salmeron et al. 1992).

Erythromycin is used in combination with Vancomycin to treat listeriosis in pregnant women (Charpentier and Courvalin 1999). All of the *Listeria* spp. that resisted Vancomycin also resisted Erythromycin (13.3%), If these isolates were to transfer from feral hogs to humans, it could result in serious consequences without the ability to treat the infection with this combination.

Streptomycin was resisted by 44.8% (n=13) of the *Listeria* spp. isolates, and resistance to this antibiotic has been traced to a gene largely spread from the genera *Enterococcus* and *Streptococcus* as well (Charpentier et al. 1995).

Lincomycin was the most common antibiotic that *Listeria* spp. resisted at a rate of 89.7% (n=26). Other studies such as Conter et al. (2007) found that Lincomycin was one of the most common antimicrobials that resisted by most strains of *Listeria* spp.

Fifty-one and one half percent of the generic *E. coli* isolates were susceptible to all antibiotics in the study, which is not unexpected when the sample size of 132 isolates from 36 total samples is taken into consideration. Also, the resistance of Sulfisoxazole easily being the highest at 43.9% is relatively expected when looking at similar studies (Branham 2007).

E. coli's resistance to Tetracycline (2.3%) could possibly be explained by its wide use in swine production as a treatment for infection. It is also used as a growth promoter in unison with many Sulphonamides, which could possibly explain why one isolate (0.8%)

exhibited resistance to both Sulfisoxazole and Tetracycline (Bischoff et al. 2002). There was one other representation of an association of a Sulphonamide's resistance in unison with another antibiotic in the study as well. The two isolates (1.5%) that exhibited this were resistant to the antibiotics Nalidixic Acid and Sulfisoxazole, but none were resistant to Nalidixic acid alone.

One isolate (0.8%) of generic *E. coli* showed a significant resistance to Ceftiofur. Ceftiofur is approved as an antibiotic for use in food animals in the United States; therefore resistance to this antimicrobial is of concern to the production animal industry. Although, Okeke et al. (2000) found two isolates of *E. coli* in humans that resisted Ceftiofur, showing that these bacteria that resist Ceftiofur in livestock have transferred *E. coli* to humans. Our find of Ceftiofur resistance in feral hogs shows that the transfer of resistance in wildlife in the area is occurring.

Sixty five percent of the *Salmonella* spp. isolates were susceptible to all antibiotics in the study, with isolates exhibiting the most resistance to Sulfisoxazole at a rate of 29.7%. This rate of resistance is not unordinary for a sulfonamide, which can exhibit a difference of 10% to 75% resistance in *Salmonella typhimurium* (Huovinen et al. 1995) and has been recorded around 47.3% in a study that also found a sulfonamide to be the most resisted antibiotic when testing isolates from humans (Helms et al. 2002).

There was one isolate of *Salmonella* spp. that exhibited resistance to Ampicillin, Cefoxitin, and Amoxicillin-Clavulanic acid. Resistance to Ampicillin and Amoxicillin-Clavulanic acid has been more common than Cefoxitin in commercial swine in other studies

(Gebreyes et al. 2000). This study, however, showed 5.4% of the isolates resisting Cefoxitin and only 2.7% resisting Ampicillin and Amoxicillin-Clavulanic acid. Cefoxitin is a second generation cephalosporin, which are commonly prescribed in hospital settings because it has a low level of major side-effects and the large number of ailments it can treat (Jewesson et al. 1983). Cefoxitin is resisted by *Salmonella* in some cases but has not been to be to the same degree as Sulfonamides, Ampicillin and Amoxicillin-Clavulanic acid (Carattoli et al. 2002).

E. coli can exchange genetic material with other pathogens such as *Salmonella*, *Shigella*, *Yersinia* and *Vibrio* (Okeke et al. 2000). Thus, the presence of antibiotic resistant *E. coli* may contribute to antibiotic resistance in other common pathogens as well.

Okeke et al. (2000) found isolates of *Escherichia coli* 0157 that came from domesticated swine exhibited the highest frequencies of resistance when compared to cattle, food, and humans; making hogs a viable threat to the spread of antibiotic resistance in pathogens. Antibiotic resistance levels were lower in the current study, which utilized feral hogs compared to Okeke's study. Reasons for the differences remain unclear; however, it is clear that feral hogs do possess pathogens that are resistant to some antibiotics.

A study Kanuganti et al. (2002) found a higher isolation rate of *Listeria* spp. in pork products compared to fecal contents of pigs. The higher rate of isolation in pork products could be due to the contamination of production facilities. Regardless of source of contamination, presence of *Listeria* spp. in the product reveals a potential for transmission

to humans. When looking at the harvesting and processing of feral hogs the same threat exists due to meat contamination which can be the result of how they are killed, dressed, handled, and processed, usually by untrained personnel (Rabatsky-Ehr et al. 2002). The potential provided by *Listeria* spp. in a meat product can be compounded if the isolate is resistant to antibiotics. *Listeria* spp. is a common food contaminant that has begun to gain many different antibiotic resistant genes (Roberts et al. 1996). This is particularly alarming because *Listeria* spp. can lead to neonatal abortion and meningitis (Farber and Peterkin 1991).

IMPLICATIONS

The findings of this study indicate that feral hogs in Texas do possess some levels of antibiotic resistant *Escherichia coli*, *Salmonella* spp. and *Listeria* spp. Coupled with the other documented viral and bacterial diseases that feral hogs can harbor, they pose a likely risk to human and veterinary health (Hutton et al. 2006). With the home range of some feral hogs as large as 70,000 acres (Taylor 2003), and the increased invasion of feral hogs into urban areas (Agrilife Extension 2008), the likelihood of the contraction of antibiotic-resistant *Escherichia coli*, *Salmonella* spp. and *Listeria* spp. is significantly increased. With these findings in mind, the options of control and/or abatement should be implemented.

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