Antibiotic Resistant Bacterial Profiles of Anaerobic Swine Lagoon Effluent

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Although land application of swine (Sus scrofa) manure lagoon effluent is a common and effective method of disposal, the presence of antibiotic-resistant bacteria, both pathogenic and commensal can complicate already understood issues associated with its safe disposal. The aim of this study was to assess antibiotic resistance in swine lagoon bacteria from sow, nursery, and finisher farms in the southeastern United States. Effluents from 37 lagoons were assayed for the presence of Escherichia coli, Campylobacter, Listeria, and Salmonella. Antibiotic resistance profiles were determined by the Kirby-Bauer swab method for 12 antibiotics comprising eight classes. Statistical analyses indicated that farm type influenced the amount and type of resistance, with nurseries and sow farms ranking as most influential, perhaps due to use of more antibiotic treatments. Finisher farms tended to have the least amount of antibiotic class resistance, signaling an overall healthier market pig, and less therapeutic or prophylactic antibiotic use. Many bacterial isolates were resistant to penicillin, cephalosporin, and tetracycline class antibiotics, while nearly all were susceptible to quinolone antibiotics. It appeared that swine farm type had a significant association with the amount of resistance associated with bacterial genera sampled from the lagoons; nurseries contributed the largest amount of bacterial resistance.

Swine concentrated animal feeding operations (CAFOs) generate approximately 5.7 L of effluent pig⁻¹ d⁻¹ (Pork Checkoff, 2008a). The CAFO swine production system is typically partitioned into three basic swine farm types including: farrowing (sow), nursery, and finisher operations, accounting for birth, weaned young piglets, and market pigs, respectively (Pork Checkoff, 2008b). Approximately 67 million pigs (domestically) are in inventory on an annual basis (National Agricultural Statistics Service, 2008) with lagoon systems as the primary means for storage and disposal of swine manure. With the recent 20-yr trend toward increased CAFO production and fewer small farm operations, effluent production can only increase and the available land to which this waste can be safely applied will be in high demand. Most swine manure is minimally treated to reduce microbial pathogens, despite some costly technology which has become available for more thorough treatment (Hill and Sobsey, 2003; Vanotti et al., 2005, 2007). Most of the available technology can and has been shown to reduce bacterial pathogens by as much as between 1 and 4 orders of magnitude (Hill and Sobsey, 2003; Vanotti et al., 2005, 2007), however very few of the studies account for antibiotic resistant bacteria (ARB), which can outnumber pathogens by many orders of magnitude, due to their presence as commensal bacterial genera or species such as E. coli, Pseudomonas, and Staphylococcus (Kelley et al., 1998; Sengelov et al., 2003; Sapkota et al., 2007).

The majority of stored lagoon effluent (the wastewater slurry comprised of fecal matter and water) is land-applied as slurry to on-farm forage or other crops (Adeli and Varco, 2001; McLaughlin et al., 2005; Read et al., 2008). Typical lagoons are approximately 2- to 4-m deep and can be several hectares in area. Storage takes place throughout the fall and winter months, while land application takes place from late spring throughout the summer months (Adeli and Varco, 2001). Anaerobic conditions, which are inherent in this type of lagoon system, not only are useful for stabilizing volatile organic compounds (VOCs), but also for reducing microbial pathogens (Hill and Sobsey, 2003). This simple type of two-step treatment system (anaerobic lagoon storage followed by land application) is useful and has served the industry well.

Lagoon systems can host a vast array of microbial constituents, but most microbes derive from deposited fecal matter and are in-
fluenced by the microbial and physicochemical constituents inherent in the swine production operation (i.e., feed, water and antibiotic inputs). Studies by Leung and Topp (2001), Sengelov et al. (2003), Chinivasagam et al. (2004), and Binh et al. (2008), demonstrated the overall microbial quality of various swine lagoon systems and the influence of antibiotics shed in feces of the different types of swine farm management systems. Sengelov et al. (2003) determined that up to 10^7, 10^6, and 10^7 colony forming units (CFU) mL^-1, respectively, of tetracycline-, erythromycin-, and streptomycin-resistant bacteria, resided in swine lagoon effluent samples collected from farms which used these respective antibiotics. Compounds used in the feed, water, or therapeutic oral and injection treatments of pigs in CAFOs can encompass a broad antibiotic class range including: aminoglycosides,cephalosporins, chloramphenicol, fluoroquinolones, macrolides, penicillins, and tetracyclines and each class can contain variants which may be prescribed for veterinary use, but may be homologous to human-approved antibiotics (Jindal et al., 2006; Rajic et al., 2006). At each stage between farrowing and finishing, these antibiotic classes may be mixed and matched to meet certain growth or therapeutic requirements and thus exert their own respective influences on the manure bacterial population (Sengelov et al., 2003; Rajic et al., 2006).

Public health can be compromised by overuse of antibiotics, as has been recently demonstrated with outbreaks of community acquired methicillin-resistant Staphylococcus aureus (MRSA) and Clostridium difficile in the U.S. and Europe (Lieberman 2003). Antibiotic over-prescription and non-judicious use has brought forth and helped establish multi-agency antibiotic resistance monitoring programs in North America (National Antibiotic Resistance Monitoring System) and Europe. In Europe, these concerns and pressures have caused the abandonment of subtherapeutic (i.e., growth promoting) antibiotic use in CAFOs, while domestically, industries such as the poultry industry have made strong commitments to reducing antibiotic use (Isaacson and Torrence, 2002).

The purpose of this study was to determine the antibiotic profiles (antibiograms) of select microbial isolates including typical fecal indicator and pathogenic bacteria from anaerobic swine manure lagoons on southeastern United States swine farms. As part of a two-part study investigating the microbial quality of swine lagoon effluent, samples were collected from 37 lagoons. Pathogens and indicators were isolated, identified, and characterized for antibiogram. Samples were collected from sow, nursery, and finisher swine farms to ascertain the influence that each respective management approach would influence onto the antibiograms of each respective population.

**Materials and Methods**

**Lagoon Sample Collection**

Samples were collected from 37 anaerobic swine manure storage lagoons in the southeastern United States. Sample collection comprised 17 sow (breeding, gestation, farrowing), 10 nursery (21 d old to 18 kg feeders), and 10 finisher (feeders grown to 113 kg) lagoons starting in the fall of 2007 and ending in spring of 2008; each site was visited once during this time period. Samples were collected in sterile 250 mL polypropylene bottles using a modified combination PVC floatation boat, pipette, and hand pump previously described for swine lagoon effluent collection (McLaughlin and Brooks, 2009). Six samples were collected per sample collection, from each lagoon, comprising two sets of three near and far house-side samples. Immediately following sample collection, bottles were placed on ice in a cooler and transported to the laboratory for analyses.

**Microbial Analyses**

Samples were processed as part of a two-part microbiological quality and characterization of swine lagoons in the southeastern United States; part one comprised the enumeration of various microbial pathogens and indicators, while part two involved antibiotic characterization of select isolates. As such, quantitative techniques were used to isolate bacterial isolates despite no quantitative data presented here. Where appropriate, enrichment techniques as part of a Most Probable Number method (MPN) were used for bacterial genera expected to be present at low concentrations. Quantitative data is presented in part one of this study (McLaughlin et al., 2009).

Samples were processed for the presence of *E. coli*, Campylobacter, Listeria, and Salmonella. *E. coli* isolates were selected from mFecal Coliform agar (mFC) (Neogen-Accumedia; Lansing, MI) plated using membrane filtration with incubation at 44.5°C for 16 h. Three random, typical thermal-tolerant (growth and lactose fermentation at 44.5°C) colonies were isolated from each sample. To confirm *E. coli* isolates, a colony polymerase chain reaction (PCR) employing primers specific for the *E. coli* uidA gene was used following previously established reaction conditions (Bower et al., 2005). A single colony was lysed by suspending in 1 mL PCR grade water (Acros-Organics; Geel, Belgium) and heating to 98°C for 10 min, followed by centrifugations at 10,000 × g for 10 min. The resulting supernatant (10 μL) was used as template in the PCR assay which consisted of 1 X PCR Buffer II (Applied Biosystems; Foster City, CA), 2.5 mmol L^-1 MgCl₂ (Applied Biosystems), 0.2 mmol L^-1 dNTP mixture (Promega, Madison, WI), 1.5U AmpliTaq Gold (Applied Biosystems), and 2 μmol L^-1 primer pair (Integrated DNA Technologies; Coralville, IA). The resulting product was qualitatively assessed by electrophoresis in a 2% agarose gel, stained in ethidium bromide, and photographed using an Alphatech gel imager (Alpha Innotech; San Leandro, CA). An *E. coli* PCR positive control consisted of *E. coli* ATCC (American Type Culture Collection) 25922 and was subjected to the entire sample process from plating onto mFC to selection and PCR amplification. Positive bands measured approximately 380 bp according to comparison with a 100 bp DNA mass standard ladder (Promega). Approximately 10% of positive PCR products were sequenced via an ABI 3730 XL DNA sequencer (Applied Biosystems) and those sequences confirmed via BLASTn analysis (Altschul et al., 1990) against NCBI Genbank sequences.

Campylobacter were enumerated and isolated from a MPN enrichment technique. The MPN was established using a three-tube by three-dilution design, typically consisting of 1.0, 0.1,
and 0.01 mL of effluent inoculated into 10 mL of Campylobacter enrichment broth (Neogen-Accumedia) and incubated at 35°C for 4 h followed by 44 h at 42°C under microaerophillic conditions. Conditions were established using the Anoxomat system (Mart Microbiology, Drachten, The Netherlands) with the preset microaerophillic gas mixture selected. Following the 48 h incubation cycle, an aliquot of each presumptively positive tube was streaked for isolation onto 5% sheep blood (Hema Resources & Supply, Willamette Valley, OR) tryptic soy agar (Neogen-Accumedia) plates and incubated microaerophillically at 42°C for 48 h. All typical Campylobacter colonies were selected and prepared as described above for colony PCR. Samples were subjected to PCR with Campylobacter-specific primers (designed for real time PCR, approximately 100 bp) targeting the 16S rRNA gene using previously established conditions (Lund et al., 2004). The PCR products were subjected to electrophoresis and the resulting product compared to a DNA mass standard as described above. Approximately 10% were sequenced as stated for E. coli. Campylobacter jejuni ATCC 33560 was used as a positive control and was subjected to the entire MPN technique and subsequent PCR amplification.

Salmonella isolates were isolated using an enrichment MPN technique in which 1.0, 0.1, and 0.01 mL of undiluted effluent were added to 10 mL of buffered peptone water and incubated for 24 h at 35°C in a water bath. Following this pre-enrichment, 0.5 mL of each tube was transferred to 10 mL of Rappaport-Vassiliadis R10 broth (Neogen-Accumedia) and incubated at 42°C for 24 to 36 h in a water bath. Aliquots from presumptive positive RV R10 broth tubes (300 μL from each tube) were spot inoculated (three droplets of 100 μL) to mSRV agar (Neogen-Accumedia) in six-well plates (Thermo Fisher Scientific-Nunc, Rochester, NY) and incubated for 24 to 48 h at 42°C. All positive mSRV plates were then probed and streaked for isolation onto Hektoen Enteric agar (Neogen-Accumedia) overnight at 42°C. All typical blue-green colonies exhibiting sulfur reduction were presumed positive and streaked to tryptic soy agar. As conducted with E. coli and Campylobacter, colony PCR, subsequent electrophoresis, and DNA sequencing (10% of PCR products) were used to determine the presence of Salmonella using primers targeting the invA gene (437 bp) using previously established conditions (Liu et al., 2002). Salmonella enterica Typhimurium ATCC 14028 was used as a positive control as described above.

Listeria were isolated using an enrichment MPN technique in which 1.0, 0.1, and 0.01 mL of undiluted effluent were added to 10 mL of Buffered Listeria enrichment broth (Neogen-Accumedia) and incubated at 30°C for 24 to 30 h. After the 24 to 30 h incubation, an aliquot of 0.1 mL from each tube was transferred to 10 mL of Fraser broth (Neogen-Accumedia) and incubated for 48 h at 35°C. Each tube displaying esculin hydrolysis was streaked for confirmation to Oxford agar (Neogen-Accumedia) and incubated at 35°C for 48 h. Typical gray colonies demonstrating esculin hydrolysis were selected as presumptive *Listeria*. Colonies were subjected to confirmation with *Listeria* genus or *L. monocytogenes* specific primers using two separate PCR assays. Before amplification, *Listeria* presumptive colonies were suspended in tryptic soy broth and incubated for 16 h at 35°C; a 1/10th dilution was prepared from this broth and subjected to lysis for 30 min at 98°C, followed by centrifugation as described above. *Listeria* genus and *L. monocytogenes* PCR and electrophoresis were performed with primers targeting the conserved *prs* *Listeria* gene (370 bp) and *hly* gene (designed for real time PCR, approximately 64 bp) using previously established conditions, respectively (Doumith et al., 2004; Rodriguez-Lazaro et al., 2004). Positive PCR products were electrophoresed and sequenced as described above. *Listeria monocytogenes* ATCC 51722 was used as a positive control as described above.

**Antibiogram**

ARB antibiograms (antibiotic resistance pattern) were characterized using the Kirby-Bauer swab method (Bauer et al., 1966). A total of no more than three typical random isolates from mFC (E. coli), Oxford (Listeria), Hektoen enteric (Salmonella), and 5% sheep blood (Campylobacter) agar were isolated from each sample. The antibiogram consisted of 12 antibiotics encompassing eight antibiotic classes: penicillin (ampicillin, penicillin), cephalosporin (cephalothin), peptide (polymixin b), glycopeptide (vancomycin), macrolide (erythromycin), aminoglycoside (neomycin, amikacin, gentamicin, kanamycin), tetracycline (tetracycline), and quinolone (ciprofloxacin). Samples were plated to either Mueller Hinton (Neogen-Accumedia) (E. coli, Salmonella, and Campylobacter) or tryptic soy agar (Listeria) in 150-mm Petri dishes and were stamped with BBL Sensi-disc (BD-BBL, Franklin Lakes, NY) antibiotics using a BBL antibiotic disc dispenser (BD-BBL, Franklin Lakes, NJ). E. coli, Listeria, and Salmonella were incubated at 35°C for 16 to 24 h, and zones of inhibition (mm) were manually measured and compared to National Committee for Clinical Standards (NCCLS) reference breakpoints for resistance zone diameters (NCCLS, 2003a, 2003b) to determine either antibiotic resistance or susceptibility. Campylobacter were incubated at 42°C for 24 to 30 h under microaerophillic conditions. Reference bacterial isolates, Staphylococcus aureus ATCC 29293 (American Type Culture Collection; Manassas, VA), E. coli ATCC 25922, C. jejuni ATCC 33560, L. monocytogenes ATCC 51722, and Pseudomonas aeruginosa ATCC 27853 were used for quality control to standardize antibiotic effectiveness.

**Statistical Analysis**

Antibiotic class resistance was determined by grouping antibiotics into eight classes and assigning either resistant (1) to at least one antibiotic in the class or susceptible (0) scores to each grouping, thus creating a binomial data set for each antibiotic class by isolate. Antibiotics were grouped into classes to eliminate the possibility of a single isolate’s resistance to multiple antibiotics of the same class and thus over inflating its resistance level. It is important to note that resistance to an antibiotic within a class does not confer resistance to all antibiotics within that respective class, just the antibiotics tested within the class. Quantified antibiotic class resistance level (QACRL) was quantified by summing binomial scores (1 or 0) for each antibiotic class for each isolate, thus generating a number for each isolate from 0 (susceptible to all classes) to 8 (demonstrating resistance
to ≥ 1 antibiotic from each class). Differences in mean QACRL (dependent variable) between swine farm type (class variable) or lagoons (class variable) within swine farm type were determined by conducting an analysis of variance for each microbial parameter or bacterial group (indicator or pathogen). The protected Fisher’s least significant difference (LSD) test was used to determine significant differences and correct for type I error. Significant associations in percentage of multi-antibiotic class resistant isolates (dependent variable) with swine farm types (F, N, and S) (independent variable) were determined using the Chi-Square analysis for each microbial parameter or bacterial group (indicator and pathogen). Isolates were classified as either resistant to 1, 2, 3, > 4, all, or susceptible to all antibiotic classes. In addition, the binomial data (dependent variable) from each antibiotic class were used in a Chi-Square analysis to determine if significant associations existed between swine type (independent variable) and each microbial parameter’s antibiotic class resistance. The SAS Enterprise Guide 4.1 (SAS Institute, Cary, NC) was used for all statistical analyses. Unless otherwise stated α = 0.05, and all differences between treatments were significant if below this level.

Results
Antibiotic Class Analyses
To determine the influence of farm type on resistance to one type of antibiotic class, Campylobacter, Listeria, Salmonella, and E. coli were analyzed via chi-square analysis. Table 1 shows the percentage of isolates resistant to an antibiotic class according to genus and swine farm. Swine farm type influenced the resistance to penicillin (Salmonella), cephalosporin (Salmonella and E. coli) glycopeptide (E. coli), aminoglycoside (Salmonella and E. coli), macrolide (Campylobacter), tetracycline (Campylobacter, Listeria, and E. coli), and quinolone (Listeria) antibiotic classes. No significant association between swine lagoon type and peptide antibiotic class resistance was demonstrated for the investigated isolates. Eight of 12 significant associations between farm type and bacterial antibiotic resistance as determined by chi-square analysis, were associated with nurseries which represented the highest percentage of resistant isolates for a given genus and antibiotic class combination (Table 1).

Isolate Multiple Antibiotic-resistance Frequencies
A total of 758 isolates from 37 swine lagoons were investigated for multiple antibiotic class resistance (Fig. 1). Multiple antibiotic class resistance was grouped into four groups comprised as resistant to: 1, 2 or 3, > 4, or susceptible to all antibiotic classes tested, then compared across swine farm type. Isolates were grouped as a single pathogen group or E. coli. Chi-square analysis determined that the swine farm type did not influence the distribution of bacteria resistant to 1 antibiotic class, while multiple antibiotic-resistant bacteria (>2 antibiotic classes) were influenced by swine farm type. Among the pathogenic bacteria, nearly 65, 58, and 95% of pathogens tested were resistant to more than two antibiotic classes for F, N, and S, respectively. Nurseries tended to have the greatest number of isolates which were resistant to four or more antibiotics, the majority of which were E. coli. Nurseries also had the largest amount of pathogens resistant to only one antibiotic class. Among all the E. coli investigated, the majority was resistant to more than two antibiotic classes, and only 28 of the 512 demonstrated resistance to only one antibiotic class. Overall there were very few isolates which demonstrated a susceptibility to all antibiotics tested, the majority of which were Listeria.

Quantified Antibiotic Class Resistance Levels
Results for QACRL and differences between swine farm types are presented in Table 2. QACRL was generated by grouping the resistance or susceptibility to each of eight antibiotic classes (as represented by the selected antibiotics) and assigning either a 1 or 0, respectively, and summing these values for each isolate. Overall swine farm types had a significant impact on the amount of antibiotic resistance. Among the three farm types, nurseries had the greatest amount of antibiotic resistance (resistance to greater than four antibiotic classes), and three out of four of the monitored bacterial genera demonstrated this pattern, while Listeria was the only genus that did not follow this trend (Table 2). When pathogens and indicators were analyzed together (i.e., all isolates), nurseries demonstrated the greatest amount of antibiotic resistance at the class level (Table 2).

Discussion
The antibiotic resistance profiles of E. coli, Campylobacter, Listeria, and Salmonella isolates from three types of swine farm lagoons were determined and differences in profiles were noted between farm types. A total of 12 antibiotics were tested, representing the eight major antibiotic classes. Future studies will focus on other genera, other antibiotics, and include the use of broth-dilution methods, since specific antibiotic inhibitory or bactericidal concentrations can also be determined.

Overall, it appeared that swine farm type influenced the antibiotic resistant profiles as determined by ANOVA and chi-square analysis. Significant associations between the antibiotic resistance of at least one genus or species investigated and swine farm type (sow, nursery, or finisher) were found for nearly all antibiotics tested. Nurseries had the highest percentage of resistant isolates when a significant association between farm type and bacterial antibiotic class resistance was determined. Meanwhile, based on analysis of variance, the overall amount of QACRL among all isolates was significantly higher in nursery farm isolates, with the exception of Listeria, which were found to be more resistant on sow farms. No isolate was resistant to all antibiotics tested and only a small percentage (11 of 758 or 1.45%) were susceptible to all antibiotics. Nursery farm isolates, on average were resistant to between four and five antibiotic classes. These differences could be most attributed to the different level and types of antibiotics being used at the nursery swine stage (Dunlop et al., 1998; Dewey et al., 1999; McEwen and Fedorka-Cray, 2002; Rajic et al., 2006). Gram-negative bacterial isolates (E. coli, Salmonella,
and \textit{Campylobacter} were predominantly found to be resistant to penicillin, cephalosporin, and glycopeptide class antibiotics, which was most likely a result of natural, intrinsic resistance associated with their respective genera. Penicillin, cephalosporin, and glycopeptide class antibiotics tend to be more effective against Gram-positive bacteria since their primary target is the peptidoglycan layer (Yao and Moellering, 2003).

Listeria \textit{(the only Gram-positive investigated)} isolates were resistant to polymixin B, a peptide class antibiotic; this may also be attributed to natural resistance associated with the genus, since this type of antibiotic is nearly exclusively used on Gram-negative bacteria (Yao and Moellering, 2003). Nearly all isolates were susceptible to ciprofloxacin, a quinolone class antibiotic approximately 20 yr in use (Lieberman, 2003). However, increased tetracycline, 

<table>
<thead>
<tr>
<th>Antibiotic class</th>
<th>Penicillin†</th>
<th>Cephalosporin</th>
<th>Glycopeptide</th>
<th>Peptide</th>
<th>Amino-glycoside</th>
<th>Macro-lide</th>
<th>Tetracycline</th>
<th>Quinolone</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Swine type} (Organism genus or species)</td>
<td>Frequency [%]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finisher (F)</td>
<td>19/21 [90.5]</td>
<td>19/21 [90.5]</td>
<td>19/21 [90.5]</td>
<td>1/21 [4.8]</td>
<td>4/21 [19.0]</td>
<td>\textbf{18/21 [85.7]}</td>
<td>9/21 [42.9]</td>
<td>0/21 [0.0]</td>
</tr>
<tr>
<td>Salmonella</td>
<td>4/13 [30.8]</td>
<td>3/13 [23.1]</td>
<td>13/13 [100.0]</td>
<td>2/13 [15.4]</td>
<td>0/13 [0.0]</td>
<td>12/13 [92.3]</td>
<td>10/13 [76.9]</td>
<td>0/13 [0.0]</td>
</tr>
<tr>
<td>\textit{Escherichia coli}</td>
<td>140/141 [99.3]</td>
<td>19/141 [13.5]</td>
<td>132/141 [93.6]</td>
<td>0/141 [0.0]</td>
<td>30/141 [21.3]</td>
<td>72/141 [51.1]</td>
<td>\textbf{140/141 [99.3]}</td>
<td>0/141 [0.0]</td>
</tr>
<tr>
<td>Nursery (N)</td>
<td>12/13 [92.3]</td>
<td>13/13 [100.0]</td>
<td>13/13 [100.0]</td>
<td>0/13 [0.0]</td>
<td>4/13 [30.8]</td>
<td>18/21 [85.7]</td>
<td>9/21 [42.9]</td>
<td>0/21 [0.0]</td>
</tr>
<tr>
<td>Salmonella</td>
<td>\textbf{17/23 [73.9]}</td>
<td>\textbf{6/23 [26.1]}</td>
<td>22/23 [95.7]</td>
<td>1/23 [4.4]</td>
<td>\textbf{10/23 [43.5]}</td>
<td>22/23 [95.7]</td>
<td>\textbf{22/23 [95.7]}</td>
<td>0/23 [0.0]</td>
</tr>
<tr>
<td>\textit{Escherichia coli}</td>
<td>174/176 [98.9]</td>
<td>\textbf{63/176 [35.8]}</td>
<td>\textbf{172/176 [97.7]}</td>
<td>1/176 [0.6]</td>
<td>\textbf{77/176 [43.8]}</td>
<td>98/176 [55.7]</td>
<td>164/176 [93.2]</td>
<td>0/176 [0.0]</td>
</tr>
<tr>
<td>Listeria</td>
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<td>1/35 [0.0]</td>
<td>34/35 [97.1]</td>
<td>0/35 [0.0]</td>
<td>1/35 [2.9]</td>
<td>\textbf{30/35 [85.7]}</td>
<td>\textbf{6/35 [17.1]}</td>
</tr>
<tr>
<td>Salmonella</td>
<td>20/33 [60.6]</td>
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<td>33/33 [100.0]</td>
<td>0/33 [0.0]</td>
<td>29/33 [87.9]</td>
<td>32/33 [97.0]</td>
<td>0/33 [0.0]</td>
<td>\textbf{30/33 [90.9]}</td>
</tr>
</tbody>
</table>

† Bold values indicate which farm type had the largest percentage of resistant isolates when a significant association between farm type and that respective bacterium’s antibiotic resistance was determined by chi-square analysis \((P < 0.05)\). Lack of a bold values indicates no significant association between the bacterium’s antibiotic resistance and farm type.

### Table 2. Mean QACRL by swine lagoon type†.

<table>
<thead>
<tr>
<th>Isolate or Group§</th>
<th>F</th>
<th>N</th>
<th>S</th>
<th>Least square difference Type‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Escherichia coli}</td>
<td>3.78</td>
<td>\textbf{4.26}#</td>
<td>3.22</td>
<td>F</td>
</tr>
<tr>
<td>\textit{Campylobacter}</td>
<td>4.24</td>
<td>\textbf{4.53}</td>
<td>3.46</td>
<td>F</td>
</tr>
<tr>
<td>\textit{Listeria}</td>
<td>1.44</td>
<td>1.22</td>
<td>\textbf{2.06}</td>
<td>F</td>
</tr>
<tr>
<td>\textit{Salmonella}</td>
<td>3.38</td>
<td>\textbf{3.45}</td>
<td>3.45</td>
<td>F</td>
</tr>
<tr>
<td>Pathogen</td>
<td>2.53</td>
<td>2.79</td>
<td>\textbf{2.96}</td>
<td>F</td>
</tr>
<tr>
<td>All</td>
<td>3.34</td>
<td>\textbf{3.83}</td>
<td>3.13</td>
<td>F</td>
</tr>
</tbody>
</table>

† QACRL—Quantified Antibiotic Class Resistance Level—sum of binomial data (resistance = 1, susceptible = 0) of each antibiotic class for each isolate or bacterial group.

‡ Swine farm type: F—Finisher, N—Nursery, S—Sow.

§ Group: Pathogen—Mean value for \textit{Campylobacter}, \textit{Listeria}, and \textit{Salmonella}.

†† N/S—Not significant.
macrolide, and aminoglycoside resistance among the *E. coli* and some of the pathogenic isolates may be indicative of their use on the visited farms. It has been hypothesized that up to 2% of a bacterial population, and up to 10% in an antibiotic-exposed population can be resistant to any antibiotic, just due to natural mutation rates (Novick, 1981), however a selective pressure (i.e., antibiotic use) may be needed to express this as a dominant genotype and subsequent phenotype. Tetracycline class antibiotics such as chlorotetracycline and oxytetracycline, and β-lactams, such as penicillin and cephalosporin have been predominantly used as veterinarian-prescribed treatments in CAFOs such as swine farms (Jindal et al., 2006; Rajic et al., 2006). The majority of antibiotic use in swine production appears to be as a prophylactic during the initial stages of the weaning nursery pigs (Rajic et al., 2006). Common antibiotic class cocktails included aminoglycoside-tetracycline, penicillin-aminoglycoside, and tetracycline-penicillin-sulfa combinations. This stage can be considered the most vulnerable time for infection, however following this stage, the amount and frequency of antibiotic use is reduced (Rajic et al., 2006).

In the livestock industry, it appears that antibiotic use is diminishing, at least at the prophylactic level (Wierup, 2001; Isaacson and Torrence, 2002). European nations have banned the use of antibiotics aimed at nontherapeutic use. While this practice has been received with some public enthusiasm, recent efforts to reduce overall use (Isaacson and Torrence, 2002). How-
ever, even after an antibiotic has ceased to be employed within an industry, some of the resistance to the antibiotic may remain hidden in the genetic code of bacterial populations as transposable elements or may be co-selected with chemical-, disinfectant-, or other antibiotic-resistant gene sequences (Rusin and Gerba, 2001; Rensing et al., 2002). Some farm environments, in which a specific antibiotic's use has been eliminated, have shown resistance rates similar to farm sites currently using the antibiotic in question (Jindal et al., 2006).

**Conclusions**

Agricultural industries have made strides toward reducing antibiotic over-prescription, but public-health concerns remain. One cannot be absolutely certain of the future public and animal health implications of antibiotic use in CAFO farm situations. Some argue that the overprescription of antibiotics by the medical industry has led to some of our most recent issues, while others blame animal agriculture (Isaacson and Torrence; 2002; Lieberman, 2003). If CAFOs are part of this problem, then the critical issue becomes one of explaining the mechanism of movement of these organisms from the farm to a public-health concern?

This study determined the presence of antibiotic-resistant bacterial populations in swine manure lagoons and determined that their presence and characteristics were most likely determined by variable selective pressures implemented at different stages of swine rearing. It appeared that nurseries more likely influenced the presence of resistant isolates, suggesting selective pressures due to management approaches and antibiotic use. While the presence of antibiotic-resistant bacteria and genes in swine effluent may be cause for concern, it is unknown what, if any, impact they have once exposed to a nonselective (no antibiotic use) environment and to harsh physicochemical, climate, and microbial variables. Addressing these issues will be the aim of future studies.

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**References**


