Incidence of Antibiotic Resistance in Fecal *Escherichia coli* Isolated from Commercial Swine Farms

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**ABSTRACT:** Sows and pigs from 10 commercial swine farms were sampled to determine patterns of resistance of fecal *Escherichia coli* to five commonly used antibiotics. Before testing, farms were categorized as high or low antibiotic use based on interviews with the respective producers. On each farm, fecal swabs were obtained from five sows at 7 d postpartum and from five pigs from each sow at 7, 35, and 63 d of age. A total of 6,296 *E. coli* isolates from 750 pig fecal samples and a total of 462 *E. coli* isolates from 50 sow fecal samples were tested against apramycin, carbox, gentamicin, neomycin, and oxytetracycline using a standardized disk diffusion test. Percentage of resistant organisms was compared between pigs at the various stages of growth, between sows and their respective pigs, and between farms of high and low antibiotic use. Incidence of resistance was greater (P < .05) in pigs at 35 or 63 d of age compared with 7 d of age for most antibiotics, and patterns differed between high-use and low-use farms. Resistance differed (P < .05) among *E. coli* from pigs compared to their respective sows for apramycin and carbox on low-use farms, whereas incidence of resistance on high-use farms differed (P < .05) between sows and pigs for apramycin and oxytetracycline. These data indicate that patterns of antibiotic resistance are dependent on age of pig and level of antibiotic use.

Key Words: Antibiotics, *Escherichia coli*, Sows, Pigs


**Introduction**

Use of antibiotics remains commonplace in U.S. livestock operations due to the therapeutic value against disease and enhanced performance of animals fed subtherapeutic concentrations (Kiser, 1976). Increasingly, however, bacterial resistance to antibiotics has caused concern among health specialists and consumer groups (Braude, 1978; Kunin, 1993; Cassell, 1995), and one focus of these concerns is the widespread use of drugs in the livestock industry. Several investigations have focused on emergence of drug-resistant bacteria (Siegel et al., 1974; Dawson et al., 1984; Langlois et al., 1984), persistence of resistant bacteria (Hinton et al., 1984; Langlois et al., 1988), and effects on human medicine (Kunin, 1993). However, little information is available with regard to resistance in modern production facilities or with regard to antibiotic products of more recent use in livestock diets. The purpose of this investigation was to determine the current incidence of resistance to commonly used antibiotics in modern commercial swine facilities.

**Materials and Methods**

Ten commercial swine farms from various regions of Tennessee were selected for the study. All farms were typical of U.S. farrow-to-finish, high-intensity swine production. Size of farms ranged from 200 to 600 sows, and all pigs were weaned between 17 and 25 d of age. Farms were divided into two types, based on antibiotic use, as determined by detailed interviews with the respective producers. Farms were classified as low-use (LU) if subtherapeutic feed-based antibiotics were not used, or if only subtherapeutic concentrations of tetracyclines were used for brief periods during disease outbreaks. Likewise, injectable antibiotics were not used except for short periods to control atypical disease outbreaks. In general, such use was limited to less than 2 wk per year. All managers of low-use farms (n = 3) indicated a conscious effort to reduce or eliminate antibiotic use, either for monetary or other unspecified reasons. Farms in the high use (HU) group (n = 7) were those where subtherapeutic...
concentrations of feed-based antibiotics were routinely used and(or) injectable antibiotics were routinely used as a preventative measure. Farms in the HU group also commonly used antibiotics on a continuous basis to control chronic disease conditions.

Five sows were randomly selected from each farm and fecal material was collected via rectal swab at 7 d postpartum using Culturette collection tubes (Becton Dickson, Cockeyville, MD). This system allows inoculated swabs to be saturated with .5 mL of Amies medium (Atlas and Snyder, 1995) for stabilization of bacteria during transport. Swabs were also obtained from five randomly chosen pigs from each test sow on the same day. Pigs were identified by ear notch so that additional samples could be collected at 35 and 63 d of age. These ages corresponded to phases in which the pigs were housed in the farrowing crate, nursery, and grow/finish units, respectively. A few test pigs were lost from production during the course of the study or could not be reliably identified at 35 or 63 d of age due to injury at or near ear notch sites. Thus, fewer pigs were sampled at 35 and 63 d of age, resulting in fewer E. coli isolates being tested in the second and third sampling periods. Due to randomized selection of animals, sows and pigs consisted of purebred and multicros animals.

Samples were maintained on ice while being transported to the laboratory. The majority of samples were processed on the same day as collection; however, it was occasionally necessary to store swabs for up to 2 d on ice due to the distance of some farms from the laboratory and the need to collect from several remote farms over successive days. To ensure that the storage period did not affect E. coli counts or characteristics, we tested bacteria from five fresh swabs and the same swabs after storage for 48 h on ice. We did not detect any differences in the number of E. coli or percentage of antibiotic resistance of isolates between fresh and stored swabs.

Upon arrival at the laboratory, swabs were immediately streaked onto lactose MacConkey agar (DIFCO, Detroit, MI) and incubated overnight at 37°C. Following incubation, 10 pink to red colonies, with colony characteristics typical of E. coli, were randomly selected from each sample for the study. In a few instances, less than 10 characteristic E. coli colonies could be found, and in those samples all such colonies were selected for the study. To confirm our ability to selectively identify E. coli, approximately 2% of test colonies exhibiting characteristics typical of E. coli and a similar number of colonies not typical of E. coli were randomly chosen from the samples by the person responsible for selection of all test isolates. That person has extensive experience differentiating enteric bacteria on a variety of media. Selected colonies were subjected to biochemical analysis (API20, Vitek bioMerieux, Syosset, NY) to identify the isolates to the species level. In all cases, colonies suspected of being E. coli were determined to be E. coli by the biochemical analysis. The majority (96%) of isolates not phenotypical of E. coli were determined to be species other than E. coli, Klebsiella spp. being the most common group. Escherichia coli isolates selected for resistance testing were restreaked onto Luria Bertani (LB) agar (Bertani, 1952), incubated overnight at 37°C, and stored at 4°C until determinations of antibiotic resistance were performed.

Resistance Determination

Within 30 d after collection, all test isolates were subjected to a standardized disk diffusion test (Bauer et al., 1966). In that test, a wire loop was used to transfer colonies to 5 mL of sterile LB broth in glass tubes. Tubes were capped with foam stoppers and incubated in an orbital shaking water bath at 37°C until turbidity matched that of a .05% McFarland standard (NCCLS, 1990). Following incubation, 150 μL of each culture was transferred to Petri dishes containing Mueller-Hinton agar (NCCLS, 1990). Cultures were spread over the surface of the media with a glass rod to obtain confluent growth and were allowed to dry for 15 min before application of antibiotic disks. All cultures were subjected to separate paper susceptibility disks (Baxter Diagnostics, McGaw Park, IL) containing 15 μg of apramycin (Eli Lilly Co., Indianapolis, IN), 20 μg of carboxad (Sigma, St. Louis, Mo.), 10 μg of gentamicin (ICN Biomedicals, Aurora, OH), 30 μg of neomycin (ICN), and 30 μg of oxytetracycline (ICN). These concentrations were recommended by the respective manufacturers for determination of sensitivity to the antibiotics. Final incubation was carried out at 37°C for 24 h. Following incubation, clear zones surrounding antibiotic disks were measured and compared to reported zones established by manufacturers of the antibiotics. Isolates were determined to be sensitive, intermediately resistant, or resistant based on their respective zone sizes. Potency of the disks was routinely tested using a control E. coli strain, ATCC 25922, which is sensitive to all antibiotics tested. In the control tests, zones of clearing were measured following inoculation and incubation as described above and compared with zone sizes reported for sensitive E. coli.

Statistical Analysis

For each antibiotic, two-way contingency tables were created either for each farm type or for each level of antibiotic usage. The two-way tables consisted of resistance level by either generation (sow or pig) or pig age (7, 35, or 63 d of age). Chi-square tests and Fisher’s Exact Test (Steel and Torrie, 1980) were used to analyze differences in patterns of resistance within each table. Cochran-Mantel-Haenszel tests (SAS, 1990b) were used if patterns differed across age, generation, or level of antibiotic use. Calculations were conducted with the SAS FREQ procedure (SAS,
Table 1. Percentage of resistant E. coli isolates from pigs of varying ages under high and low antibiotic use

<table>
<thead>
<tr>
<th>Item</th>
<th>Day 7&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Day 35</th>
<th>Day 63</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HU&lt;sup&gt;c&lt;/sup&gt;</td>
<td>LU</td>
<td>HU</td>
</tr>
<tr>
<td>n&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1,731</td>
<td>747</td>
<td>1,683</td>
</tr>
<tr>
<td>Apramycin</td>
<td>8.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>9.2&lt;sup&gt;f&lt;/sup&gt;</td>
<td>60.9&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carbadox</td>
<td>15.1&lt;sup&gt;i&lt;/sup&gt;</td>
<td>18.7&lt;sup&gt;j&lt;/sup&gt;</td>
<td>57.0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>73.9&lt;sup&gt;k&lt;/sup&gt;</td>
<td>81.7&lt;sup&gt;k&lt;/sup&gt;</td>
<td>92.0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Neomycin</td>
<td>55.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>44.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>54.8&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>96.2&lt;sup&gt;e&lt;/sup&gt;</td>
<td>82.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>94.4&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data derived from a standardized disk susceptibility test.
<sup>b</sup>Age of pig.
<sup>c</sup>HU = farms with high antibiotic use (n = 7); LU = farms with low antibiotic use (n = 3).
<sup>d</sup>Number of E. coli isolates tested within each category. All isolates were tested against each antibiotic.
<sup>e</sup>, <sup>f</sup>, <sup>g</sup>, <sup>h</sup>Percentages with unlike superscripts within day and antibiotic are different at <sup>e</sup>, <sup>f</sup>, <sup>g</sup>, <sup>h</sup>P < .001 or <sup>i</sup>, <sup>j</sup>, <sup>k</sup>, <sup>l</sup>P < .05, based on chi-square and Fisher’s Exact Test.

1990a) and CATMOD (SAS, 1990b). Isolates determined as intermediate resistant were analyzed as a separate category and as part of the sum containing resistant and intermediate resistant isolates. Because we considered intermediate resistance to be a degree of resistance for the purpose of this discussion, intermediate resistant isolates were combined and are presented in this paper as a part of the total resistant colonies.

Results

With the exception of oxytetracycline, the percentage of resistant E. coli was higher (P < .001) on HU farms for all antibiotics in pigs at 35 and 63 d of age, compared with LU pigs (Table 1). Resistance to oxytetracycline was not different (P > .05) between farm types at 7 d of age. Greater incidence of resistance occurred in LU pigs only at 7 d of age, when incidence was observed to be higher for carbadox and gentamicin in LU pigs than in HU pigs (P < .05 and P < .001, respectively). Although significant, these differences were not great in contrast to much larger differences generally observed between LU and HU pigs at 35 d of age. We can offer no explanation why a greater percentage of resistant isolates occurred in LU pigs for those two antibiotics at that time.

High-use farms were observed to have the greatest incidence (P < .001) of resistant E. coli to apramycin (60.9%), carbadox (57.0%), and gentamicin (92%) in pigs at 35 d of age compared with 7 and 63 d of age using the Cochran-Mantel-Haenszel test for the appropriate interaction. In these same pigs, the greatest incidence of resistance to neomycin (64.6%) and oxytetracycline (98.8%) occurred at 63 d of age (P < .001). In contrast, there were no differences (P > .05) in the percentage of resistant E. coli between age groups on low-use farms for apramycin or carbadox. The LU pigs had a greater incidence of resistant E. coli to gentamicin (81.7%) and neomycin (44.6%) at 7 d of age (P < .001), whereas greatest resistance to oxytetracycline in E. coli from LU pigs occurred at 63 d of age (P < .001).

At 7 d after farrowing, LU pigs had greater (P < .001) percentages of resistant E. coli to apramycin (9.2%) than their respective sows (.7%; Table 2). Incidence of resistance was greater to carbadox (38.6%, P < .001) and neomycin (53.6%, P < .05) in sows than in pigs (18.7 and 44.6%, respectively) in the LU group. No differences (P > .05) were observed between LU sows and pigs for gentamicin or oxytetracycline. For the HU group, incidence of resistance was also greater (P < .001) in pigs than in sows for apramycin (8.2 and .3%, respectively). In contrast to the LU group, incidence of resistance to oxytetracycline was greater (P < .05) in HU sows (99.0%) than in their pigs (96.2%). No differences (P > .05) were observed between HU sows and pigs for carbadox, gentamicin, and neomycin.

Discussion

Even though farms were separated into low-use and high-use categories, based on interviews with producers, more specific information cannot be presented with regard to detailed antibiotic regimens for each farm. Although all producers indicated strict adherence to label indications of the drugs (Table 3), a number of producers could not reliably recall detailed drug-use history beyond a few weeks before the initiation of the study. Additionally, a few producers were not aware that some purchased feeds used on their farm contained antibiotics, as was noted upon subsequent observation of feed tags. Thus, we felt more specific indications were not reliable and might result in erroneous conclusions. Only those farms where little or no antibiotic use could be confirmed for more than 1 yr previous to the study
were included in the LU group. Thus, we are confident that the data presented here are a conservative indication of differences between these two farm types.

Resistance to oxytetracycline was high on nearly all farms for all phases of growth. This probably reflects 1) tetracycline's long history of use in the swine industry; 2) no mandated withdrawal time, resulting in this antibiotic’s frequent use throughout the growing/finishing period and in sow diets; and 3) high persistence of tetracycline resistance following use (Langlois et al., 1986). The extensive and long-term use of oxytetracycline and chlortetracycline have apparently resulted in large populations of tetracycline-resistant bacteria in swine as well as in other species (Turtura et al., 1990; Allen et al., 1993).

Even though the incidence of resistance to most antibiotics was similar between sows and their respective pigs at 7 d after farrowing, some differences did occur. Resistance to apramycin and carbadox was higher in E. coli isolated from pigs than in E. coli isolates from their respective sows. Both of these antibiotics are commonly used to control scours and a variety of other problems associated mainly with young pigs. Apramycin is commonly used in the nurseries as a feed additive and for postweaning medication; thus, these data may indicate some transfer of organisms may be occurring from nurseries to farrowing rooms. If that is the case, the contamination seems to have a greater effect on the bacterial populations in young pigs than in sows. This may be due to resistance being carried by bacteria that specifically colonize young pigs, or it may be due to a greater resistance by the sow to invasion from bacteria from an outside source. However, a greater percentage of E. coli isolated from sows were resistant to oxytetracycline compared to isolates from their respective pigs. Once again, this may be because tetracycline is used more extensively in older animals and sows have a longer period of exposure to tetracycline than do pigs.

We initially hypothesized that resistant isolates in young pigs were the result of transfer of bacteria from the sow. However, these data indicate that other young pigs may be a more likely source of resistant isolates, with transfer occurring as early as 7 d of age. This would support other work (Callear and Smith,

<table>
<thead>
<tr>
<th>Item</th>
<th>High use</th>
<th>Low use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sows Pigs</td>
<td>Sows Pigs</td>
<td>Sows Pigs</td>
</tr>
<tr>
<td>ndd</td>
<td>322</td>
<td>1,731</td>
</tr>
<tr>
<td>apramycin</td>
<td>.3e</td>
<td>.7e</td>
</tr>
<tr>
<td>Carbadox</td>
<td>17.7</td>
<td>15.2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>74.8</td>
<td>73.9</td>
</tr>
<tr>
<td>Neomycin</td>
<td>56.2</td>
<td>55.5</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>99.1</td>
<td>96.2</td>
</tr>
</tbody>
</table>

*d,a,b Data derived from a standardized disk susceptibility test.
*b,d Data are from seven farms with high antibiotic use.
*c Data are from three farms with low antibiotic use.
*Number of E. coli isolates tested within each category. All isolates were tested against each antibiotic.
*e,f,g,h Percentages with unlike superscripts within day and antibiotic are different at e,f $P < .001$ or g,h $P < .05$, based on chi-square and Fisher’s Exact Test.

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Table 3. Label indications of tested antibiotics for swine a

<table>
<thead>
<tr>
<th>Antibiotic Indication</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apramycin (F)b</td>
<td>For control of porcine colibacillosis (weanling pig scours).</td>
</tr>
<tr>
<td>Carbadox (F)</td>
<td>For control of swine dysentery (vibronic dysentery, bloody scours or hemorrhagic dysentery); control of bacterial swine enteritis salmonellosis or necrotic enteritis caused by Salmonella choleraesuis; to increase rate of weight gain and improve feed efficiency.</td>
</tr>
<tr>
<td>Gentamicin (F, W, I, O)</td>
<td>For control of colibacillosis in pigs and weanling swine; control of swine dysentery caused by Treponema hydrosentariae.</td>
</tr>
<tr>
<td>Neomycin (F, W)</td>
<td>For control of bacterial enteritis including scours caused by E. coli and Salmonella spp.</td>
</tr>
<tr>
<td>Tetracycline (F, W, I)</td>
<td>Bacterial enteritis caused by E. coli and Salmonella choleraesuis and pneumonia caused by Pasteurella multocida; to increase weight gain and improve feed efficiency.</td>
</tr>
</tbody>
</table>

*Summary of a minimum of two manufacturer labels for each antibiotic.
*b = feed additive, W = water additive, O = oral, I = injectable.
1966; Arbuckle, 1968) suggesting that pigs, and not the sow, are the primary source of fecal E. coli in farrowing barns. Additionally, Hinton and Linton (1987) and Katouli et al. (1995) determined that young pigs were able to maintain their own unique population of microflora, even while in close contact with the sow and littermates. Other investigators also observed higher percentages of resistant bacteria in young mammals than in adults (Linton et al., 1972; Sogaard, 1973; Wierup, 1975; Langlois et al., 1986). Langlois et al. (1986) postulated that bacteria from young animals may have a higher percentage of resistance because E. coli can more easily and rapidly colonize the intestinal tract of younger individuals. Additionally, Larsen and Larsen (1972) and Wierup (1975) suggested that resistance may be greater in young animals because bacteria in their intestinal tract have the increased potential for resistance transfer.

Significant changes in the percentage of resistant E. coli were observed over time, especially in the HU group. In pigs, the percentage of isolates that were resistant to apramycin, carboxax and gentamicin increased rapidly from 7 to 35 d of age. In most cases, producer records confirmed increased drug use at the time of weaning, and this practice was likely a primary factor in the increased resistance observed in the 35-d-old pigs. It was also noted that the greatest differences in resistance between HU and LU farms occurred in pigs at 35 d of age. In most cases, therapeutic use of drugs decreased in the grower phase, and this was reflected in stable or reduced resistance at 63 d of age. However, subtherapeutic use of tetracyclines was common during this phase in HU farms, and this was reflected by the fact that tetracycline resistance increased from 35 to 63 d of age, in contrast to resistance to other antibiotics.

Because most pigs remained healthy throughout this study and determinations of pathogenic isolates were not conducted, we cannot speculate on how resistance affected health of the animal and(or) efficacy of potential therapeutic regimens. It might be suggested that performance benefits associated with subtherapeutic use may be compromised by large-scale resistance. However, Smith (1967) and Visek (1978) disputed this theory by showing resistance to antibiotics had risen with increased use, whereas associated performance benefits had remained constant over the same number of years. However, those authors did not address the progressive production and use of new antibiotics that may have maintained the performance advantage while resistance increased with older drugs. Even though a direct link between performance benefits and antimicrobial effects on microflora has not been convincingly shown, many theories suggest at least indirect involvement of the microflora in performance advantages attributed to antibiotics (Visek, 1978; Roth and Kerchgesnern, 1993). If these theories are correct, then resistance, which by definition limits antibacterial effects, should also limit the impact of antibiotics on performance benefits. Rigorous studies that compare the rate of resistance acquisition by the major enteric species to measures of performance will be essential to determine the true impact of resistance on animal production.

Implications

This study indicates widespread resistance to commonly used antibiotics within typical swine herds. Additionally, bacteria from farms where antibiotic use is limited have significantly lower incidence of resistance, except in the case of tetracyclines, for which resistance is widespread. Because resistance may affect later therapeutic or subtherapeutic value of those antibiotics, management strategies to reduce resistance acquisition by bacteria may be warranted.

Literature Cited


