Antibiotic resistance patterns and genotypes of *Salmonellae* within swine production systems and the relationship to on farm use of antibiotics.

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Abstract: A total of 206 *Salmonella* isolates were obtained from fecal samples from swine and environmental sites and tested for antibacterial resistance. The most common resistances were to tetracycline, sulfamethoxazol, and streptomycin. Some isolates were resistant to as many as 9 antibiotics in the test panel. However, 31% were sensitive to all antibiotics, 15% were resistant to a single antibiotic, 30% were resistant to two antibiotics and 20% were resistant to 3 antibiotics. Salmonella isolates from the same farm that had the same pattern of antibiotic resistances also had the same PFGE genotype and serotype.

Keywords: Antibiotics, resistance, genotype, PFGE, tetracycline.

Introduction: The usage of antibiotics for both therapeutic and growth promotion purposes in swine has been linked to the emergence of antibiotic resistance in *Salmonella enterica*. The emergence of resistant strains of *S. enterica* has been considered a significant threat to public health. By determining the distribution of antibiotic resistances in *S. enterica* isolated from healthy swine and on farm usage of antibiotics, it was hoped that we could estimate the importance of the current antibiotic usage in swine production on the emergence and distribution of antibiotic resistance.

Materials and Methods: Fecal and environmental samples were collected during the summer months from 11 Illinois swine farms. Each sample was subjected to a double enrichment protocol designed for the isolation of *S. enterica* (Isaacson, et al, 1999). A total of 206 *S. enterica* isolates were obtained. All isolates were tested for antibiotic resistance using an automated microdilution technique. The antibiotics used to screen for resistance and the specific dilutions used were based on the National Antimicrobial Resistance Monitoring System (NARMS). Seventy-
two of the *S. enterica* isolates were selected to determine genotype and serotype. The genotype data was from pulsed-field gel electrophoresis (PFGE). Serotyping was performed by the National Veterinary Services Laboratory.

**Results:** The most common resistances were to tetracycline (67.4 %), sulfamethoxazol (51.9 %), and streptomycin (21.6 %) (see figure 1). Resistance to ampicillin, chloramphenicol, several cephalosporins, and trimethoprim also were detected and to a lesser extent aminoglycosides and 4-fluoroquinolones (including nalidixic acid). 31% of the isolates were sensitive to all antibiotics (figure 2). Most strains (65 %) that were resistant had phenotypic resistances to between 1 and 3 antibiotics: 15% were resistant to a single antibiotic, 30% were resistant to two antibiotics and 20% were resistant to 3 antibiotics. Some isolates were resistant to as many as 9 antibiotics in the test panel.

As part of our broader study on the epidemiology and transmission of *S. enterica* in swine, 72 isolates were selected for genotyping using PFGE (refer to R. Weigel in these proceedings). The results demonstrated that within a single farm several PFGE profiles existed. However, when genotype and antibiotic resistance patterns within a single farm were compared, strains with the same antibiotic resistance pattern had the same PFGE profile. Isolates from two (or more) different farms that had the same antibiotic resistance patterns did not share the same PFGE patterns. These results suggest that within a farm that common isolates based on PFGE and antibiotic resistance pattern were clones. Similarly, when serotype was added as another distinguishing trait, there again was complete concordance between serotype, PFGE type, and antibiotic resistance pattern within a farm but not between farms.

Tetracycline was used as feed a feed additive on 9 of the 11 farms and all farms had *S. enterica* that were resistant to tetracycline. The range of resistance isolates among the farms was from 9-100 %. The two farms that reported not using tetracycline had fewer resistant strains (16.6 % and 20 %). However, the farm with the lowest prevalence of tetracycline resistant *S. enterica* (9 %) indeed used tetracycline (but only for 1 week in the production cycle). If the usage patterns were divided into 3 groups; no use, use for 1-2 weeks, and use for greater than 2 weeks, the tendency was that the longer the use, the more *S. enterica* that were resistant to tetracycline (table 1). However, this was not without exceptions. In the 1-2 week use group, the range of resistances was from 9-98%. Thus, usage patterns could not accurately predict the prevalence of tetracycline resistance.
Discussion: The results demonstrated, as expected, wide spread dissemination of antibiotic resistances among the *S. enterica* strains isolated. The most common resistances were against tetracycline, streptomycin, and sulfamethoxazole. These antibiotics are older drugs that have been in use for decades. The results also demonstrated that within a farm there was a concordance between antibiotic resistance pattern, PFGE genotype, and serotype but that among different farms, the same antibiotic resistance pattern did not correlate with the same genotype or serotype. Tetracycline has been used as a feed additive since the 1950’s. Thus, it was expected that there would be widespread resistance to this antibiotic. Indeed, this was the most commonly detected resistance. There was a tendency that the greater the usage of tetracycline on farm, the greater the prevalence of resistance. It was noted that this was not a hard and fast rule. Furthermore, it should be pointed out that almost a third (32.6 %) of all *S. enterica* isolates were sensitive to tetracycline, despite its wide spread use. It is interesting that despite wide spread resistance in *S. enterica*, tetracycline remains an effective growth promotant.

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References

Table 1. Use of tetracycline

<table>
<thead>
<tr>
<th>Usage pattern</th>
<th>Per cent of isolates resistant/farm</th>
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<tbody>
<tr>
<td>No use</td>
<td>20%, 16.6%</td>
</tr>
<tr>
<td>1-2 weeks of use</td>
<td>65.3%, 9%, 96.5%, 98%</td>
</tr>
<tr>
<td>Greater than 2 weeks of use</td>
<td>100%, 50%, 100%, 100%, 100%</td>
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Figure 1

Figure 2