Salmonella prevalence, serotypes, and patterns of antimicrobial resistance in cohorts of nursery and finishing pigs

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Introduction

Salmonella are now being monitored by the Food Safety Inspection Service of the USDA for purposes of process control in slaughter plants, and as a sentinel organism for emerging antimicrobial resistance (1). Public concerns about the emergence of foodborne bacteria with multi-resistant phenotypes, and about the use of antimicrobials in food animals, are increasing. Despite considerable research, the epidemiology of asymptomatic Salmonella in pigs is poorly understood (2). The purpose of this paper is to examine the association between nursery prevalence of Salmonella and finisher prevalence, and to describe patterns of antimicrobial use and antimicrobial resistant Salmonella in modern swine rearing facilities.

Materials and methods

In 17 cohorts of pigs raised in multiple-site production systems, we determined prevalence of fecal shedding and serotypes of Salmonella in both nursery and finishing phases. For each cohort of approximately 600 to 1000 pigs, individual fecal samples were collected from 96 pigs within 1 week of being transferred from the nursery site (9-10 weeks of age), and before the first cut of pigs for slaughter (approximately 25 weeks). For each cohort, the floor of the finishing barn was also sampled after disinfection and before pig placement using drag swabs (10 per barn). Samples were cultured for Salmonella using conventional enrichment procedures, and serotypes were determined by the NVSL, Ames. All isolates per group were tested for antimicrobial resistance except for those where more than 15 isolates of a single serotype were obtained. In these cases, a subset of 15 isolates selected randomly of the respective serotype was tested. Isolates were tested for resistance to 11 antimicrobials: amikacin(Ak), amoxicillin/clavulanic acid(AC), ampicillin(Am), cefotaxime(Cf), cephalexin(Cph), chloramphenicol(Cm), ciprofloxacin(Cp), gentamicin(Gm), piperacillin(Pl), tetracycline(Tr), and trimethoprim/sulfur(TrS). MIC values were determined using the Biometerix Vitek Jr. computerized microbiological identification, susceptibility and data management system, using ATCC 25922 E. coli as a quality control strain. NCCLS break points were used to differentiate resistant from susceptible isolates (3,5). Percent of resistant isolates to each antimicrobial agent was determined by dividing the number of isolates to the particular antimicrobial by the total number of isolates tested.

Results

Salmonella prevalence and serotypes:

The most frequently isolated Salmonella serotypes were typhimurium (copenhagen), typhimurium, and derby (2). In the nurseries, Salmonella was detected in 14 out of 17 (82 %) groups, and the prevalence of fecal shedding of Salmonella ranged from 0% to 64% (median 7%). A total of 13 different serotypes were isolated from nursery groups, with the most prevalent (highest to lowest) being typhimurium (copenhagen), typhimurium, derby, infantis, and anatum. More than one serotype of Salmonella was isolated from 14 of 19 (93%) positive nursery groups (median 3 serotypes per site, maximum 6 serotypes). In the finishing phase, Salmonella was detected in 16 of 17(94%) groups, and prevalence ranged from 0% to 44% (median 10%). Again, multiple serotypes were isolated in most (11/16) positive groups (median 2 serotypes, maximum 4 serotypes). The total number of different finisher serotypes was 11 and the most prevalent serotypes were typhimurium (copenhagen), schwarzengrund, typhimurium, derby, heidelberg, and worthington. Using simple linear regression, prevalence of fecal shedding in nursery pigs was not associated with prevalence in finishing (p=0.91; R^2=0.00). Salmonella was detected in drag swabs of floors on 14/17 (82%) finishing farms. In 5 cases, finisher pigs shed a Salmonella serotype that had been found on drag swabs, but not in nursery pigs.

Antimicrobial resistance:

Salmonella isolated from fecal samples showed resistance to multiple antimicrobials in all positive cohorts of pigs except one (15/16). We have found resistance to a total of 6 antimicrobials out of the 11. Highest resistance was seen to tetracycline (98%) and lowest to gentamicin (5%). All fecal isolates tested were sensitive to amikacin, cefotaxime, cephalexin, ciprofloxacin and trimethoprim/sulfur. We found resistance to cephalexin and trimethoprim/sulfur in our new study under progress increasing the total to 8 (unpublished).

Among serotypes, resistance was found most frequently with typhimurium (copenhagen) (37%), typhimurium (31%), worthington (25%), derby (18%), and infantis (13%).
<table>
<thead>
<tr>
<th>Serotype</th>
<th>Total</th>
<th>Am(%)</th>
<th>Cm(%)</th>
<th>Te(%)</th>
<th>AmCmTe(%)</th>
<th>Gm(%)</th>
<th>AmCmTeGm(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhimurium</td>
<td>142</td>
<td>126(89)</td>
<td>91(64)</td>
<td>132(93)</td>
<td>91(64)</td>
<td>1(1)</td>
<td>1(1)</td>
</tr>
<tr>
<td>(copenhagen)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typhimurium</td>
<td>73</td>
<td>71(97)</td>
<td>9(12)</td>
<td>72(99)</td>
<td>9(12)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Derby</td>
<td>49</td>
<td>14(29)</td>
<td>13(26)</td>
<td>49(100)</td>
<td>12(24)</td>
<td>14(29)</td>
<td>12(24)</td>
</tr>
<tr>
<td>Heidelberg</td>
<td>23</td>
<td>0</td>
<td>2(9)</td>
<td>23(100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Interestingly, the most prevalent serotypes also exhibited the highest proportion of resistance. *Salmonella typhimurium (copenhagen)* was the most prevalent isolate (36% of all isolates) and had the highest rate of resistance (37%), followed by *Salmonella typhimurium* (20% prevalence, 31% resistance). Out of the total 330 fecal isolates tested, 86 (26%) exhibited resistance to five or more antimicrobials of the 11 tested. 64% of *typhimurium (copenhagen)* isolates fell in this category. The most common pentaresistant pattern exhibited by *typhimurium (copenhagen)* was amoxicillin/clavulanic acid, ampicillin, chloramphenicol, piperacillin, and tetracycline. Other serotypes showing pentaresistance were derby, typhimurium, and worthington.

*Salmonella typhimurium* definitive type 104 (DT104) is currently of major concern due to its zoonotic importance and resistance to five antimicrobial agents: ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline (4,6). We have found 100 (47%) of total 215 *Salmonella typhimurium* (including *copenhagen*) isolates with resistant patterns consistent with DT104, since these isolates showed resistance to ampicillin, chloramphenicol and tetracycline (Table 1). This three resistance pattern was seen in 13 of 17 (76%) nursery farms and 8 of 17 (47%) finishing sites.

**Discussion**

The prevalence of *Salmonella* in the nursery didn’t predict *Salmonella* prevalence in market-age hogs. The existence of multiple serotypes at most sites and the disparity between serotype profiles at the nursery and finishing stages indicate a dynamic pattern of *Salmonella* infection and shedding in growing pigs. Results of the antimicrobial resistance testing indicate a high level of resistance to commonly used classes of antimicrobials and some classes that are not used in swine including chloramphenicol which is possibly attributable to the involvement of mobile DNA elements coding for multiple resistance, such as integrons (7). The addition of tetracycline to animal feeds has been implicated as a stimulus for the emergence of resistance to tetracycline. The data is consistent with the contention that serotypes showing multiple resistance may be more likely to become established in growing pigs. As public concern of antibiotic resistance in human pathogens such as Salmonella continue to mount, it is essential for swine practitioners to make knowledgeable recommendations to producers about the proper use of antimicrobial agents. Improved understanding of *Salmonella* epidemiology, and the impact of antimicrobial use in swine on the emergence of resistance is required.

**References**

2. Davies, P.R., Bovee, F.G., Funk, J., Morrow, M., Jones, F. 1998. Isolation of *Salmonella* serotypes from feces of pigs raised in a multiple site production system. JAVMA 212: 1925-1929
3. NCCLS. 1991. MIC Interpretive standards of Three Categories of Susceptibility for Organisms Other than *Haemophilus and Neisseria gonorrhoeae*; NCCLS 11:17