RESEARCH REPORT

PORK SAFETY

Title: On-farm antimicrobial use and patterns of antimicrobial resistance of *Salmonella* isolates collected on farms and at slaughter plants.
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I. Abstract

Salmonella is an important foodborne pathogen that can exhibit resistance to multiple antimicrobial agents. We evaluated antimicrobial resistance in 370 *Salmonella* isolates obtained from pigs at 5 farms and 486 isolates from these pigs after commercial slaughter. Samples of feces from defined groups of finishing pigs on commercial farms, and cecal and mesenteric lymph node samples from the same groups of pigs after slaughter were cultured for *Salmonella* and tested for resistance to a panel of 11 antimicrobials. We found that the prevalence of antimicrobial resistance varied among serotypes, the most striking example being the occurrence of multiple resistance in 94% of isolates of *S. typhimurium*, compared with 11% of isolates of all other serotypes. Concordance between *Salmonella* serotypes from on-farm samples and those serotypes isolated after slaughter varied widely among farms, suggesting that risk of exposure to *Salmonella* during transport and lairage remains a concern under contemporary industry conditions. Slaughter plant studies based on bacterial culture do not provide a reliable index of the *Salmonella* status of commercial swine farms nor their associated patterns of antimicrobial resistance.

II. Introduction

Salmonella has been the preeminent foodborne pathogen worldwide for many decades, with swine identified as an important source of *Salmonella* in some countries. *Salmonella* and other enteric foodborne pathogens, particularly Campylobacter, are also implicated as vehicles for the transmission of antimicrobial resistance from food animals to humans. To define links between antimicrobial use on farms and the emergence of antimicrobial resistance in foodborne pathogens, researchers need reliable measures of antimicrobial resistance at the population (herd) level. Obtaining herd samples at slaughter plants has logistical advantages, but potential cross-infection of pigs with *Salmonella* from non-farm sources during transport and lairage casts doubts on the reliability of data on antimicrobial resistance of isolates collected at slaughter.

Galton et al. (1953) stated that the major problem of swine salmonellosis was the pig's exposure to contaminated holding facilities during the last hours of life. Subsequent studies also found a higher (2- to 4-fold) proportion of pigs infected with *Salmonella* in lairage or after slaughter than on farms (Kampelmacher et al., 1963; Newell and Williams, 1971). Possible explanations were: 1) cross-infection from other pigs or the environment during transport and lairage; and 2) increased excretion of *Salmonella* by infected pigs due to "stress". Both explanations are reasonable, and their relative importance may depend on factors such as the duration of transport and the standard of the slaughter facilities (Newell and Williams, 1971). For epidemiologic studies, one worker concluded that collecting samples at slaughter could not replace sampling at farms because of the risk of infection after leaving the farm (Newell and Williams, 1971). The fact that lymph node and intestinal cultures can be positive for *Salmonella* within hours of exposure (Fedorka-Cray et al., 1995) underlines the potential for cross-infection, beyond the farm gate, to confound studies at slaughter plants. However, this question has never been investigated quantitatively nor under contemporary conditions of transport and slaughter in the U.S. swine industry.

Several current studies of *Salmonella* in swine in the U.S. include collection of samples at commercial slaughter plants (NPPC), but reliability of these data remains suspect. We will relate on-farm antimicrobial use with patterns in farm and slaughter isolates, and use antimicrobial resistance patterns and *Salmonella* subtyping to define,

under contemporary commercial conditions, the relative importance of post-farm infection of pigs with *Salmonella*.

III. Objectives

1. Relate histories of antimicrobial use in groups of pigs to patterns of antimicrobial resistance in *Salmonella* isolates obtained from the same pigs on farms and at slaughter plants.

2. To evaluate, under contemporary commercial conditions, the importance of post-farm infection with *Salmonella* by comparing farm and post-farm isolates using a) serotypes, b) patterns of antimicrobial resistance, and c) subtypes determined by pulse-field gel electrophoresis.

IV. Procedures

Study Design

Salmonella isolates were obtained in 2 independent studies: Study 1 comprised 2 cohorts of pigs on each of 4 farms. Two of the herds (farms 1 and 2) were on farrow-to-finish farms on which the finishing barns were managed by continuous flow. Farms 3 and 4 were part of multiple-site systems in which the finishing barns were managed all-in/all-out (AIAO). Each cohort comprised 60 recently weaned pigs. Sampling of the pigs on the farms was repeated at approximately 15 weeks of age, 22 weeks of age, and within 48 hours before transport to slaughter, and fecal samples were assessed for the presence of *Salmonella*. Pigs were transported 10 to 24 hours before slaughter, held overnight in lairage, and killed as the first group the next day. At slaughter, mesenteric lymph nodes were collected from the region of the ileocolic junction and cecum, and an approximately 3 cm square was resected from the wall of each cecum. Study 2 involved one barn of approximately 1200 pigs (40 pens x 30 pigs/pen) housed in one finishing barn (AIAO) at a finishing site in a multiple-site production system. Before commencing the study, the presence of *Salmonella* in the chosen group of pigs was confirmed by culture of 50 fecal samples collected from pen floors in the nursery facility. Pen prevalence was estimated by bacteriologic culture of feces collected with a gloved hand from 21 pigs (of approximately 30 pigs) in each of 36 pens (total of 756 pigs), and again 21 days later in 30 of the same pens, 14 days before the first pigs were sent to slaughter. Within pens, pigs were sampled by convenience and were not individually identified. The pigs were marketed in groups of 60 (10 pigs from 6 pens) over 15 days of commercial slaughter, but samples were not recovered from one group because of a breakdown at the plant. The ceca were incised with a sterile scalpel blade, and approximately 10g of cecal contents were collected.

Bacterial culture and selection of isolates

Separate protocols for culture of fecal samples for *Salmonella* were used in study 1 (Fedorka-Cray et al., 1998) and study 2 (Davies et al., 1998), but methods were uniform within studies. For each pig in study 1, individual lymph nodes were passed through a flame and placed in a sterile petri dish until 10g of lymph node tissue were obtained. The nodes were then sliced with a sterile scalpel blade and transferred to a sterile plastic bag to which approximately 90 ml of buffered peptone water (BPW) was added. Similarly, BPW (9:1 w/w) was added to the bags containing the cecal tissue and contents. Following pre-enrichment in BPW overnight at 37°C, 1 ml was added to 9ml of tetrathionate and GN Hajna broths and the samples were subsequently processed in the same manner as the fecal samples (Fedorka-Cray et al., 1998). In study 2, cecal

contents were processed the same as fecal samples collected on farms (Davies et al., 1998). Isolates with colony morphology consistent with *Salmonella* were screened by conventional biochemical methods before serotyping at the National Veterinary Services Laboratories, Ames, Iowa. Antimicrobial resistance testing was performed on all isolates from Study 1. For Study 2, resistance testing was conducted on 162 isolates from on-farm fecal samples and 165 isolates from cecal cultures after slaughter. Slaughter isolates included all viable strains of serotypes other than *S. derby* and *S. typhimurium* var. *copenhagen*. For these two most prevalence serotypes, up to 5 isolates per serotype were selected for each day of slaughter. When more than 5 isolates of a given serotype were recovered on a day, 5 of the isolates were randomly selected for antimicrobial resistance testing.

Antimicrobial resistance testing

Isolates were tested for resistance to a panel of 11 antimicrobials: amikacin(Ak), amoxicillin/ clavulanic acid (Ax), ampicillin (Am), cefotaxime (Cf), cephalothin (Ce), chloramphenicol (Cl), ciprofloxacin (Cp), gentamicin (Ge), piperacillin (Pi), tetracycline (Te), and trimethoprim/sulfamethoxazole (TS). MIC values were determined using the Biomerieux Vitek Jr. computerized microbiological identification, susceptibility, and data management system, using ATCC 25922 *E. coli* as a quality control strain. NCCLS break points (National Committee on Clinical Laboratory Standards, 1991) were used to differentiate resistant from susceptible isolates. When MIC values were equal to NCCLS breakpoints, the isolates were deemed to be susceptible, so as not to overestimate the frequency of resistance.

V. Results

Antimicrobial Resistance of Salmonella *Isolates*

These studies yielded 858 isolates comprising 27 *Salmonella* serotypes. The most common resistance observed among these isolates was to tetracycline (80% of all isolates), with resistance to ampicillin (42%), chloramphenicol (31%), amoxicillin/clavulanic acid (30%), and piperacillin (29%) also commonly seen. Resistance to multiple antimicrobials was also frequently observed, with 44% of the isolates resistant to more than one antimicrobial.

Of the 856 isolates tested, 159 (19%) were susceptible to all 11 antimicrobials tested. The remaining isolates exhibited one of 23 different resistance patterns. The most common pattern, found in 37% of strains, was resistance to tetracycline alone. Surprisingly, the second most common pattern, observed in 225 isolates (26%), was pentaresistance to AxAmClPiTe. There was also a striking association of resistance pattern with serotype. For example, of the 225 isolates exhibiting the AxAmClPiTe resistance phenotype, 220 (98%) were *S. typhimurium* var. *copenhagen*, and 86% of isolates of this serotype had this pattern. Similar examples of resistance patterns being associated with individual serotypes were found for three other prevalent serotypes. For *S. derby* and *S. heidelberg*, isolates resistant to tetracycline alone comprised 77% and 91% of all isolates of these respective serotypes. For *S. typhimurium*, 58% of isolates were resistant to ampicillin and tetracycline, while an additional 20% were resistant to those two antimicrobials and to pipericillin.

The four most frequent serotypes, *S. typhimurium* var. *copenhagen*, *S. derby, S. typhimurium*, and *S. heidelberg*, comprised 80% of all isolates, and 46% of these were resistant to more than one antimicrobial. Multiresistance was observed in 95% of *S.*

typhimurium isolates (including *copenhagen*). In contrast, for isolates of the remaining 23 serotypes, 70% were sensitive to all antimicrobials and an additional 25% were resistant to tetracycline alone, and only 3% exhibited resistance to two or more antimicrobials. One interpretation of these data is that serotypes that acquire multiple resistance determinants gain a selective advantage that allows them to predominate in the face of antimicrobial usage on farms.

Isolation of Salmonella *on Farms and at Slaughter*

To examine whether patterns of antimicrobial resistance in isolates collected at slaughter plants corresponded with patterns observed in isolates from pigs on the farms of origin, we compared these two sets of isolates in each study. The first study comprised two cohorts of pigs on each of four farms. Across all cohorts, 202 *Salmonella* isolates (11.5% of 1790 fecal samples cultured) were obtained from pigs on farms and 315 isolates (41.3% of 810 cecal or lymph node samples cultured) were obtained from the same pigs at slaughter The proportion of positive fecal samples among positive cohorts ranged from 2.1% (5 of 235) to 42.7% (97 of 227), and the number of serotypes isolated from positive cohorts ranged from 2 to 5.

We isolated *Salmonella* from all cohorts at slaughter, with the prevalence of positive samples ranging from 0.08% (1 of 118 samples) to 78.7% (74 of 94 samples), and we found multiple serotypes (3 to 7) for all cohorts of slaughtered pigs, apart from one cohort 2 of one farm, from which only 1 isolate was obtained. At the cohort level, the proportion of culture positive slaughter samples was associated with the proportion of positive fecal samples on farm (R^2 = 0.54; P = 0.03), but there was considerable discordance between serotypes found on farms and those detected at slaughter. Across all cohorts, there were 8 instances in which a serotype was detected from farm samples but not slaughter samples, 28 instances in which serotypes were detected in slaughter samples but not farm samples, and 12 instances where the same serotype was isolated from both sets of samples in a cohort.

We next sought to determine whether *Salmonella* isolates from a specific site, either cecum or mesenteric lymph node, were more representative of those found from the same animals on farms. The proportion of cecal samples positive for *Salmonella* (185 of 405 samples; 47.7%) was greater (P<0.001) than the proportion of positive lymph nodes (130 of 405; 35.2%), and in all cohorts more serotypes were isolated from cecal samples (1 to 7, median 5) than from mesenteric lymph node samples (0 to 4, median 2.5) from the same pigs. Among the 7 cohorts from which isolates were obtained from both ceca and lymph nodes, in only one cohort was the predominant serotype (*S. typhimurium* var. *copenhagen*) the same for both lymph node and cecal samples. Among 6 of these cohorts, *Salmonella* were also isolated from fecal samples of pigs at the farms within 24 hours before slaughter. The predominant serotype among lymph node isolates was the same as the predominant serotype isolated from the pigs before transport to slaughter in 5 of these 6 cohorts, and the antibiotic resistance pattern in each case was identical. In contrast, the predominant serotype among isolates from cecal samples corresponded with the predominant fecal serotype in only 1 of the 6 cohorts.

Among the most prevalent serotypes (*S. typhimurium copenhagen, S. derby*), there was an apparent association between serotype and sample origin (lymph node vs. cecum). Of 85 isolates of *S. derby* obtained at slaughter from 6 cohorts, 66 (78 %) were from cecal samples, and the number of cecal isolates exceeded the number of lymph

node isolates in all 6 cohorts. In contrast, of 119 isolates of *S. typhimurium* var. *copenhagen* among 6 cohorts, 45 (38%) were from cecal samples. However, in 3 cohorts, cecal isolates (total 20) exceeded lymph node isolates (1). In these 3 cohorts, *S. typhimurium* var. *copenhagen* had not been isolated from pigs at the farm, and these isolations may reflect recent infection during transport or lairage. In the other 3 cohorts (25 cecal vs. 73 lymph node isolates), *S. typhimurium* var. *copenhagen* had been the most common or second most common isolate from fecal samples on farms, consistent with longer term infection of the pigs.

We also isolated *Salmonella* from the floors of trucks either before loading (2 instances) or after transport (5 instances) of the pigs to slaughter. Three of the serotypes isolated post-transport had been isolated at the farm and were also isolated from the pigs after slaughter. In the remaining post-transport instances, the serotypes had not been isolated from fecal samples at the farm, but were isolated from the pigs after slaughter. In both instances where *Salmonella* were isolated from the trucks prior to loading of the pigs, the serotypes had not been isolated at the farms. These results indicate that *Salmonella* can be acquired during transport, and support the view that assessment of on-farm *Salmonella* infection by sampling at slaughter is unreliable.

The second study involved sampling of one barn of approximately 1200 finishing pigs as well as the nursery that supplied those pigs. *Salmonella* were isolated from 168 of 1436 (11.7%) fecal samples collected at the nursery or finishing farms, and from 505 of 830 (60.8%) cecal samples collected from the same group of pigs at slaughter. A total of 8 serotypes were found on farms and 14 after slaughter. Three serotypes were isolated from fecal samples collected on farm but not from cecal samples after slaughter; 9 were isolated from the ceca but not the fecal samples; and 5 serotypes were isolated from both sets of samples. However, of all isolates from ceca, 94% were of serotypes corresponding with serotypes isolated at the farm. *Salmonella derby* was the predominant serotype among fecal isolates (58% of all isolates), while *S. typhimurium* var. *copenhagen* was predominant (49%) among cecal isolates following slaughter, with *S. derby* being the next most common (32%).

Conclusions and Future Directions

This work shows that *Salmonella* isolates obtained from slaughter plants may not accurately reflect the types or numbers of *Salmonella* present on farms, due to the possible infection of pigs during transport and lairage. Therefore, studies of pre-harvest food safety that investigate farm factors should take samples from those farms, rather than using slaughter samples as surrogates.

This work also shows that antimicrobial resistance among *Salmonella* strains isolated from swine is common, including resistance to multiple antimicrobials. Further, multiple resistance is more often found among the most commonly isolated serotypes, suggesting that the use of antimicrobials might provide a selective advantage to resistant strains. This conclusion, however, must be tempered, since other factors, such as the adaptation of particular serotypes to living in pigs, should be considered. To further investigate the question of resistance and the use of antimicrobials, long-term studies incorporating the sampling of facilities over time must be conducted. We are currently engaged in such studies, and hope to elucidate the environmental factors that promote resistance and the means by which resistance genes are transferred between strains.

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