Three-Year Trend in Antimicrobial Resistance and Genotypes among *Salmonella* in Swine and Humans

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**Summary:** The aim of this study was to determine antimicrobial resistance among *Salmonella* isolated from swine and humans in North Carolina, compare genotypes among isolates from humans originated from pig-producing areas and characterize important genes. Resistance to 9 and 11 of the 12 antimicrobial agents tested was detected among isolates from swine and humans respectively. Frequency of resistance to tetracycline and b-lactams was significantly higher among isolates from swine than humans (p <0.05). Two common multi-drug resistance (MDR) patterns were found among isolates from apparently healthy swine: AmKmStSuTe and AmCmStSuTe. However, the former MDR pattern was rare among clinical isolates. Genotyping revealed that two predominant genotypes, one composed of clinical isolates and the other non-clinical were noticed. Further characterization using *Salmonella* plasmid virulence; spvA gene also revealed that this gene is absent among the most common MDR pattern, AmKmStSuTe, in swine.

**Keywords:** pig, antibiotic, fingerprint, food safety, zoonosis

**Introduction:** North Carolina is the second largest pork-producing state in the United States with more than 10 million pigs marketed each year. Pigs are known to be important reservoirs of *Salmonella* and the use of antimicrobials in food animal production has been implicated as one of the most important source of resistant organisms among human pathogens (Barber et al., 2003). Recent trends also showed that multi-drug resistant strains of *Salmonella* are common among salmonellae from pigs (Gebreyes, 2000; Farrington et al., 1999). Though antimicrobial resistant salmonellae are common among pigs in the region, no human salmonellosis outbreak in humans has been attributed to swine or pork in the last decade in the US. The aim of this study was, therefore, to determine antimicrobial resistance patterns and show whether there is phenotypic and genotypic similarities among isolates from swine and humans and among isolates collected from clinical and non-clinical settings.

**Materials and Methods:** A total of 888 *Salmonella* isolates of four serovars including Typhimurium, Heidelberg, Muenchen and Newport, from clinical human cases were retrieved from the North Carolina State Laboratory of Public Health (NCSLPH) between the period of 2000 and 2003. In swine, 1873 isolates from on-farm studies (non-clinical) and 65 clinical isolates were included in the study. Antimicrobial susceptibility testing was done using Kirby-Bauer disk diffusion for 12 antimicrobial agents using NCCLS standards [M31-A2]. The antimicrobials, respective codes used in this manuscript and breakpoints for resistance were amikacin [Ak] (64 mg/ml), amoxicillin/ clavulanic acid [Ax] (32 mg/ml), ampicillin [Am] (32 mg/ml), cefotaxime [Cf] (32 mg/ml), cephalothin [Ce] (32 mg/ml), chloramphenicol [Cm] (32 mg/ml), ciprofloxacin [Cip] (2 mg/ml), gentamicin [Gm] (16 mg/ml), piperacillin [P] (32-64 mg/ml), tetracycline [Te] (16 mg/ml) and trimethoprim-sulfamethoxazole [Tr/Su] (80 mg/ml). Pulsed-Field Gel Electrophoresis (PFGE) was used for genotyping isolates from human and swine collected at different settings: clinical and non-clinical. The specific protocol used for genotyping was as recommended previously (Gautom, 1997). Further molecular characterization was done by using polymerase chain reaction (PCR) of *Salmonella* plasmid virulence, spvA gene.

**Results:** Frequency of resistance to the most commonly used antimicrobials such as tetracycline and b-lactams was higher among isolates from swine than humans (p <0.05). However, the spectrum of resistance was wider among isolates from humans as indicated by the fact that resistance to 11...
of the 12 antimicrobials was detected including ceftriaxone and ciprofloxacin. Among swine isolates, resistance to nine of the antimicrobial agents was detected. The frequency of resistance to each antimicrobial is as shown in figure 1. Comparison of serovar composition and antimicrobial resistance frequency of human isolates from pig-producing (eastern part of the state) and non pig-producing areas (western part of the state) of the region did not reveal any phenotypic difference in composition of serovars nor frequency of antimicrobial resistance (p > 0.1). While more than 80% of isolates from humans remained susceptible to all antimicrobial agents tested, subsets of the remaining, particularly, Typhimurium isolates exhibited AmCmStSuTe resistance pattern. Among isolates from swine (non-clinical on-farm samples), two distinct penta-resistance patterns, AmKmStSuTe and AmCmStSuTe, were common. On the other hand, clinical isolates from swine also showed AmCmStSuTe pattern predominantly similar to human isolates but not AmKmStSuTe pattern. Further characterization using genotypic approaches also confirmed that the clinical isolates, irrespective of the host, were closely related (Figure 2). Analysis of Salmonella plasmid virulence, spvA gene using PCR revealed that this gene is absent among the predominant on-farm strains with AmKmStSuTe resistance pattern but present among the AmCmStSuTe ones (Figure 3).

Discussion and Conclusions: Frequency of antimicrobial resistance among Salmonella isolates from swine was higher than those from humans. This finding is consistent with reports of previous studies (Cruchaga et al., 2001). One important aspect of the findings, however, was detection of ceftriaxone and ciprofloxacin resistance among isolates from humans but not from swine. One potential explanation for this finding may be the high and possibly irrational use of these antimicrobials in humans or could also be due to sampling error. These findings also show that there is strain distinction between the two hosts. Serovar Typhimurium was the predominant serovar in both host species. However, further phenotypic characterization revealed that the most common MDR pattern in apparently healthy pigs, AmKmStSuTe, is absent among human isolates and clinical swine isolates. This may have two
implications. First, it implies that there is distinction in subtypes between the two hosts. This may further imply that other sources may also be important. Recently, report by Barber et al. stressed the underestimation of the role of non-food animal sources such as humans and pets (Barber et al., 2003). Second, the finding may indicate that only certain subtypes of this serovar are able to cause clinical illness strengthening the notion that not all strains of Salmonella serovar Typhimurium are capable of causing clinical illnesses.

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References:

ANTIMICROBIAL AGENT SUSCEPTIBILITY OF CAMPYLOBACTER AND SALMONELLA FROM SWINE HERDS WITH VARIOUS THERAPEUTIC REGIMENS

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Summary: Fecal samples were collected in pens from 27 farrow-to-finish swine herds using (n = 10) and not using fluoroquinolones (n = 17) and in herds using (n = 20) or not (n = 7) tetracycline. Workers on the farms participated to the study. Salmonella was found in 4 out of the 27 sampled herds and were all resistant to tetracycline and susceptible to enrofloxacin. No Salmonella and no Campylobacter were isolated from human. C. coli was found in all sampled herds with an average of 68.5 % positive pens. In farms not using quinolones and tetracycline, no resistance was observed among 2 herds but resistance to enrofloxacin (71 %) and to tetracycline (100 %) were observed in some farms. In herds where tetracycline was used, resistance levels varied from 7 % to 100 %. Resistance levels to quinolones in herds using this agent varied from 0 % to 100 %.

Keywords: resistance, agar dilution, fluoroquinolones, tetracycline, antimicrobial use

Introduction: Campylobacter and Salmonella are zoonotic pathogens frequently recovered from pigs. The incidence of antibiotic resistance of Campylobacter and Salmonella isolates increased over the past few years. The use of antibiotics in food animal productions would be in part responsible for the increased resistance among foodborne bacterial pathogens (McEwen & Fedorka-Cray, 2002).