Summary
Pork accounts for 25% of domestic meat consumption and non-typhoidal Salmonella remains a very important foodborne pathogen in pork. The objectives of this study were to determine antimicrobial susceptibility among Salmonella isolates from swine and humans, to conduct phenotypic analysis and determine secular trends of multi-drug resistant (MDR) types in swine. The most common and public health important serovar Typhimurium (including var. Copenhagen) were found to be commonly multi-drug resistant (MDR) with two distinct pentar-resistance types (R-types): Ampicillin, Chloramphenicol Streptomycin, Sulfamethoxazole and Tetracycline (AmCmStSuTe, 36.1%) and Ampicillin, Kanamycin, Streptomycin, Sulfamethoxazole and Tetracycline (AmKmStSuTe, 44.6%). During the three-year study, there was a significant decline of AmCmStSuTe resistance type among S. Typhimurium (including var. Copenhagen) whereas a significant increase in the AKSSuT resistance type was observed (p<0.05). More than 80% of the isolates from human subjects (n=215) were susceptible to all antimicrobial agents tested. None of the MDR types among human diagnostic specimens exhibited AmKmStSuTe pattern (the most common resistance type in swine). The discordance in resistance types between the two hosts is currently under investigation.

Introduction
According to the economic research service of the U.S. Department of Agriculture (USDA), the cost of foodborne Salmonellosis in the U.S. is estimated to be $2.4 billion per year (www.ers.usda.gov). Pork accounts for 25% of domestic meat consumption and associated products have been implicated as origins of foodborne outbreaks in the nation and globally (Molbak et al., 1999; Pontello et al., 1998; McGuire et al., 1993). The U.S. pork industry has been a net exporter since 1995. In addition, with increasing total meat production every year (84 billion pounds projected for this year alone with 1% increase from 2001), the demand for safe pork is also increasing. Salmonella remains a very important foodborne pathogen partly since public health important serotypes and multi-drug resistant strains are common in swine.

Previous studies of swine Salmonellosis in North Carolina revealed a pig prevalence of up to 48% (Funk et al., 2000). Our group also conducted a risk factor analysis and antimicrobial resistance. The findings on the former study indicate biosecurity measures including number of personnel per farm and presence or absence of on-site hygienic facilities as some of the significant factors associated with increased Salmonella shedding in pigs. Previously, we also characterized important molecular determinants of MDR in distinct Salmonella strains (Gebreyes et al., 2000; Gebreyes and Altier, 2002). The objectives of this study were to determine antimicrobial susceptibility among Salmonella isolates from swine and humans, to conduct phenotypic analysis and determine secular trends of multi-drug resistant (MDR) types in swine.
Materials and Methods

*origin of isolates*

*Salmonella* isolates from three different sources were included in this study. The first is non-clinical samples from a longitudinal study of Salmonellosis in North Carolina (Funk et al., 2001). For this study, 484 *S. Typhimurium* (including var. Copenhagen) were included. The second was 215 *Typhimurium* diagnostic human isolates from North Carolina Public Health Laboratory Services. The human isolates were derived from counties with high concentration of swine farms.

*antimicrobial susceptibility*

Antimicrobial susceptibility to 12 antimicrobial agents was determined as recommended by the National Committee for Clinical Laboratory Standards (NCCLS). Two methods were primarily used to determine antimicrobial susceptibility: Vitek Jr. (Biomerieux) and Kirby-Bauer disk diffusion methods. We tested susceptibility to ampicillin (Am), chloramphenicol (Cm), streptomycin (St), sulfamethoxazole (Su), tetracycline (Te), amoxicillin/clavulanic acid (Ax), cephalothin (Cf), ceftriaxone (Cro), ciprofloxacin (Cip), kanamycin (Km), amikacin (An) and gentamicin (Gm). Isolates with intermediate resistance to ceftriaxone were subjected to E-test for final confirmation. Quality control strains were tested routinely as recommended by NCCLS.

Results and Discussion

The most common and public health important serovar Typhimurium (including var. Copenhagen) were found to be commonly multi-drug resistant (MDR) with two distinct penta-resistance types (R-types): Ampicillin, Chloramphenicol Streptomycin, Sulfamethoxazole and Tetracycline (AmCmStSuTe, 36.1%) and Ampicillin, Kanamycin, Streptomycin, Sulfamethoxazole and Tetracycline (AmKmStSuTe, 44.6%). Among 156 Typhimurium isolates 4 (2.6%) exhibited the former penta-resistance pattern and 113 (72.4%) exhibited the latter. Whereas, among total of 328 serovar Copenhagen isolates, 171 (52.1%) exhibited the former pattern and 103 (31.4%) exhibited the latter penta-resistance pattern. The secular distribution of these two patterns in the three-year study period is shown in Figure 1.
During the three-year study, there was a significant decline of AmCmStSuTe resistance type among S. Typhimurium (including var. Copenhagen) whereas a significant increase in the AKSSuT resistance type was observed (p<0.05). This shift of strains has been seen among S. Typhimurium var. Copenhagen isolates while Typhimurium isolates consistently showed the latter resistance type. Similar observations were noted by the National Antimicrobial Resistance Monitoring System (NARMS) study since 1997 (NARMS, 1997). There has not been any recorded change in management and ecologic factors during this study period. This shift of MDR strains is explained by its association with specific genetic determinants (not shown here).

Similar MDR types were found to predominate among animal isolates tested by the National Antimicrobial Resistance Monitoring System (NARMS). Isolates with this R-type were very rare among diagnostic samples. The human arm of NARMS, which conducts surveillance on diagnostic human *Salmonella* samples also found consistently low frequency of this R-type in comparison to the clinically more common AmCmStSuTe R-type. According to the NARMS human report of 2000, among the total of 303 Typhimurium isolates tested, 27.7% exhibited AmCmStSuTe R-type, whereas only 9.2% exhibited AmKmStSuTe R-type. This difference could be attributed to the difference in carriage of important virulence factors described elsewhere (Gebreyes et al., unpublished).
Figure 2. Antimicrobial resistance profiles of *S. Typhimurium* (including var. Copenhagen) isolates from human (n=215) and swine [non-clinical] (484). Swine isolates have been found to be commonly pentaresistant. Resistance to ceftriaxone (Cro) has been exhibited in isolates from human.

Analysis of antimicrobial susceptibility among *Salmonella* isolates derived from human diagnostic specimens revealed that more than 80% of the isolates were susceptible to all antimicrobial agents tested (Figure 2). None of the MDR types among human diagnostic specimens exhibited AmKmStSuTe resistance type but 20 isolates (out of 33 MDR) in Typhimurium from humans and 11 of 15 of swine exhibited AmCmStSuTe R-type. Though the finding could be partly explained by sampling bias, the observation is interesting since the AmKmStSuTe R-type is the most common in swine and most of the human isolates were derived from swine producing counties (east of I-95). The national average reported in NARMS 2000 indicated that 49.5% (ranging between 35 and 77% per state) of Typhimurium are pan-susceptible (NARMS, 2000), which is lower than our findings (80%). This could be due to several reasons including the lower number of antimicrobials that we tested and the lower sample size that we used. One interesting finding among human isolates is the occurrence of ceftriaxone (third generation cephalosporin) resistance in two of the isolates tested. In contrast, we have not found third generation cephalosporin resistance among isolates from swine (non-clinical and diagnostic).

**Implications**

Antimicrobial resistance to different classes of antibiotics is common in *Salmonella* isolates from swine. In addition, distinct MDR strains commonly predominate among the most common serovar, Typhimurium. However, the abundance of such strains in apparently healthy swine may not directly show the human health hazard as specific resistant strains may be common in food animals but may not have clinical significance in humans. Confirmatory analysis of such discordance using genetic characterization is underway.

**Literature Cited**


Funk JA. Davies PR. Gebreves W. 2001. Risk factors associated with *Salmonella enterica*