EVALUATION OF PHENOTYPIC AND GENOTYPIC APPROACHES AS PREDICTORS OF SALMONELLA STRAINS OF CLINICAL AND NON-CLINICAL ORIGIN

Paul M. Dorr*, Daniel A. Tadesse, Wondwossen Gebreyes
*Department of Population Health and Pathobiology, North Carolina State University – College of Veterinary Medicine, 4700 Hillsborough St., Raleigh, NC, 27606. Ph: 01-919-513-8291; E-mail: wagebrey@ncsu.edu

Summary
This study investigated the possible phenotypic and genotypic similarities and differences between Salmonella isolates obtained from swine with clinical salmonellosis to isolates obtained from swine showing no clinical disease. Phenotypic analysis was done by antimicrobial resistance profiling and amplified fragment length polymorphism (AFLP) fingerprinting was employed for genotypic analysis. A total of 281 (140 clinical and 141 non-clinical) were included in this study. There was no association between the origin of isolates (clinical or non-clinical) and the antimicrobial resistance profiles with OR of 0.77 and 95%CI (0.46-1.27). However, there was association between AFLP profile and origin of isolates (distinct genotypic clustering) between Salmonella strains of clinical and non-clinical origin with an odds ratio of 8.7 and 95% CI (4.8-15.9). Therefore, we concluded that a high resolution DNA fingerprinting approach such as AFLP may be helpful in differentiating strains of primary clinical importance.

Introduction
The prevalence of Salmonella in today’s swine production systems continues to be a threat to the health of both swine and human consumers in the United States. With the emergence and persistence of multi-drug resistant (MDR) isolates in the last few decades, the threat has become even greater. Current estimates of Salmonella prevalence typically range from approximately 13% to 25% (Hurd et al., 2002; Davies et al., 1997; Rostagno et al., 2004). Many, if not a majority of these animals act as carriers and show no outward signs of clinical salmonellosis. In addition to the swine harboring and shedding Salmonella, there are many other risk factors present on and around the farm that increase this risk of infection (Funk et al., 2001). Some studies have shown a link between virulence and antimicrobial resistance (Navarre et al., 2005) while others failed to demonstrate a link between these two phenotypes under experimental conditions (Allen et al., 2001). On the other hand, the use of molecular epidemiological approaches has recently emerged as important components that may be useful for distinguishing Salmonella strains. Their use, however, as important predictors of clinically significant strains have not yet been elucidated. The purpose of this study was to compare the phenotypic and genotypic profiles of isolates obtained from primary clinical salmonellosis cases in swine to isolates obtained from clinically normal swine in North Carolina and evaluate the significance of phenotypic (based on antimicrobial resistance pattern) and genotypic (based on AFLP fingerprinting) approaches as predictors of strains that originated from clinical specimens.

Materials and Methods
Clinical Salmonella isolates were obtained from two different laboratories. All isolates were from clinical salmonellosis cases in North Carolina. The total number of clinical isolates included in the present study was 140. Of these, ninety were donated from the NCDA diagnostic laboratory. These represent clinical cases from multiple swine production systems throughout North Carolina. Additional 50 clinical isolates originated from Lab 2 that represent clinical cases from multiple farms in one production system. Non-clinical isolates (n=141) were obtained from previous and ongoing prevalence studies within the lab and were used as a convenience sam-
Amplified Fragment Length Polymorphism (AFLP) fingerprinting was carried out on all the 141 isolates as described previously (Gebreyes et al., 2005). Purified DNA was adjusted to a concentration of 10ng/µl nuclease free water (Fisher Scientific, Fair Lawn, New jersey, USA). We used a modification of the AFLP protocol first described by Vos et al (1995). Briefly, the DNA was digested with EcoRI and MseI enzymes mix (LI-COR® Biosciences, Lincoln, NE, USA) at 37°C for 2 hours and incubated for 15 minutes at 70°C. Then adapters were ligated to the restriction fragments using T4 DNA ligase at 20°C for 2 hours. The ligation mixtures were diluted 1:10 using TE buffer (LI-COR® Biosciences, Lincoln, NE, USA) and fragments were amplified using EcoRI and Msel primers (Integrated DNA Technologies, Coralville, IA, USA) for 20 cycles at 94°C for 30 seconds, 56°C for 1 minute, and 72°C for 1 minute. Selective amplifications were performed on a 1:10 diluted pre-amplification template using Msel (Integrated DNA Technologies, Coralville, IA, USA) and IRDye 800 labeled EcoRI (LI-COR® Biosciences, Lincoln, NE, USA) primer with an additional adenine at EcoRI 3’ end to obtain optimum band for analysis. Fragments were visualized on LI-COR 4200 DNA sequencer using 6.5 % KB PLUS™ polyacrylamide denaturing gel (LI-COR® Biosciences, Lincoln, NE, USA).

Statistical analysis was done using Odds ration (OR computation for association between phenotype or genotype and origin of isolates and a 95% confidence interval (CI) was computed to determine the variability and statistical significance of the association.

Results Out of the twelve antimicrobials tested for overall resistance pattern analysis, Ceftriaxone (Cro) was the only antibiotic that showed a significant difference in resistance between the clinical and nonclinical isolates. Phenotypic variability was also analyzed between the clinical and nonclinical isolates using the penta-resistance pattern common to the Salmonella phage type DT104, ACSSuT. No difference in this pentaresistance pattern was found between the clinical and non-clinical isolates (OR 0.77 , 95%CI 0.46, 1.27).

For the AFLP analysis, a cutoff of 70% was used to identify major clusters as recommended previously (Gebreyes et al., 2005). A total of 17 clonal types were identified. Using this fingerprinting approach, we detected a clear distinction between clonal types of clinical and non-clinical origin. A subset of this is demonstrated on Figure 2. As shown in the figure, two clusters predominantly of clinical origin: 1A (14 of 17 were clinical) and 1b (17 of 21 were clinical) and three clusters of predominantly isolates of non-clinical origin: cluster 2 (all five were from non-clinical source), cluster 3 (all eight were from non-clinical source) and cluster4 (12 of 13 were of non-clinical origin) were identified. There was a statistically significant association between AFLP profile and origin of isolates (clinical or non-clinical) with an odds ratio (OR) of 8.7 and 95% CI (4.77, 15.86).

Discussion The vast phenotypic similarities interspersed between the clinical and non-clinical isolates may indicate that Salmonella strains antimicrobial resistance may not be associated with the occurrence of Salmonella strains in clinical illnesses. Furthermore, this strengthens the argument that the difference in virulence between these isolates is multi-factorial and may not be strictly linked to antimicrobial resistance. Differences in production systems (1, 2, and 3 site production), environmental stressors, health status (PRRS, PCV positive herds), and nutrition may all play an important role in the expression of clinical salmonellosis in these swine herds. On the other hand, there was a lot of genotypic similarity within the clinical and non-clinical isolates. This may indicate that the use of AFLP could be a good predictor of Salmonella strain that are most commonly associated with clinical illnesses. This is a preliminary study that used convenience samples. Further work with this data including various host, pathogen and environmental factors is needed.

References
Figure 2. Dendrogram showing AFLP fingerprinting analysis of 64 Salmonella isolates from clinical and non-clinical sources.