Molecular Epidemiology of Salmonella enterica and Subtyping Using Phenotypic and Genotypic Approaches

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Summary: The aim of this study was to evaluate the discriminatory power of two phenotyping and three genotyping methods commonly used to subtype Salmonella in swine and other hosts. We found AFLP and PFGE to have the highest and comparable discriminatory power to each other. Among the 202 isolates analyzed in this study, using AFLP, 16 cluster types of S. Typhimurium were identified. Vertical spread in the production chain, from nursery to finishing farms and vertical as well as horizontal spread among finishing farms appeared to be important means of Salmonella serovar Typhimurium dissemination in swine units.

Keywords: fingerprint, food safety, salmonellae, phage type, antibiotic resistance

Introduction: Diversity among salmonellae has been studied using various phenotypic methods including serotyping, antibiotyping and phage typing, and by genotypic methods including pulsed field gel electrophoresis (PFGE) (Schwartz and Cantor, 1984), amplified fragment length polymorphism (AFLP), plasmid profiling, ribotyping, and other related methods (Gebreyes, 2003). Genotyping of Salmonella serovars is becoming an increasingly important epidemiological tool that aids in identifying sources of infection during outbreaks, detecting cross transmissions, recognizing particular strains, and monitoring intervention strategies. The aim of this study was to evaluate the discriminatory power of two phenotyping and three genotyping methods and to recommend standard approaches for epidemiological applications.

Materials and Methods: All 202 isolates of Salmonella enterica serovar Typhimurium included in this study originated from the longitudinal study described previously (Funk et al., 2001). Antibiotyping was done using two methods: Vitek Jr. (Biomerieux, Hazelwood, MO) and Kirby-Bauer disk diffusion methods as recommended by the National Committee for Clinical Laboratory Standards. Serotyping and phage typing was performed by the National Veterinary Services Laboratories (NVSL) in Ames, Iowa. PFGE, AFLP and repetitive extragenic palindromic PCR (Rep-PCR) genotyping methods were used as described previously (Gebreyes, 2003). DNAfingerprints were analyzed using the Bionumerics software (Applied Maths, Kortrijk, Belgium). We applied Simpson’s index of diversity (DI) to compare the discriminatory power of the five subtyping systems used in this study.

Results: We analyzed the discriminatory power of the five subtyping methods (AFLP, PFGE, Rep-PCR, antibiotyping and phage typing) to characterize 202 isolates. We found AFLP and PFGE to have the highest and comparable discriminatory power with a DI value of 0.939 and 0.925 respectively (Table 1). The lowest discriminatory power was observed by the Rep-PCR technique. The latter technique did not appear to discriminate within serovar when using the universal primers (UPRIME-B1) recommended by the manufacturer. As demonstrated in figure 1-A, using the Rep-PCR approach, phenotypically distinct isolates (with different resistance pattern and phage types) appeared to have similar genotypes. In contrast, as demonstrated in figure 1-B and 1-C, AFLP and PFGE were able to discriminate within distinct phenotypes of serovar Typhimurium (Figure 1). Using the standards proposed previously (Gebreyes, 2003), among the 202 isolates analyzed in this study, 16 cluster types were identified by using AFLP. Vertical spread in the production chain, from nursery to finishing farms
and vertical as well as horizontal spread from nursery and among finishing farms appeared to be important means of *Salmonella* serovar Typhimurium dissemination in swine production units.

Table 1. Subtyping methods included in this study and discriminatory index values of each.

<table>
<thead>
<tr>
<th>Subtyping Method</th>
<th>No. of types</th>
<th>Size (%) of Largest type</th>
<th>Discriminatory index (DI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFLP</td>
<td>16</td>
<td>12</td>
<td>0.939</td>
</tr>
<tr>
<td>PFGE</td>
<td>Performed on subsets</td>
<td></td>
<td>0.925</td>
</tr>
<tr>
<td>REP-PCR</td>
<td>Performed on subsets</td>
<td></td>
<td>0.421</td>
</tr>
<tr>
<td>Antibiotyping</td>
<td>12</td>
<td>56</td>
<td>0.579</td>
</tr>
<tr>
<td>Phage typing</td>
<td>9</td>
<td>55</td>
<td>0.628</td>
</tr>
</tbody>
</table>

Figure 1. Illustrations of genotyping of *Salmonella enterica* serovar Typhimurium isolates using the three genotyping methods described. 1-A. Rep-PCR; 1-B. AFLP and 1-C. PFGE (Reprinted with permission from the Journal of Swine Health and Production, American Association of Swine Veterinarians).
Discussion and Conclusions: Among genotyping methods, PFGE has been shown to have superior discriminatory power and reproducibility. Currently, it is the gold standard fingerprinting technique used for subtyping foodborne bacterial pathogens in humans under the pulsenet system (CDC). AFLP has also, in recent years, gained popularity due to its high discriminatory power and reproducibility. However, these methods are also known to have a high initial cost and moderate ease of use (Gebreyes, 2003). Based on phenotypic classification of isolates in the present study, the most common and widespread cluster type (cluster type 11) was composed predominantly of phage type DT104. The genetic diversity within DT104 in the present study was limited as compared to other phage types. This is consistent with previous reports. Using antibiotic resistance and phage typing as an alternative approach, two predominant pentaresistance types among 12 antibiotypes have been detected: AmCmStSuTe and AmKmStSuTe patterns (Gebreyes and Altier, 2002). The use of phage typing in addition to genotypic approaches remains an important step in subtyping Salmonella, particularly in outbreaks involving serovars Typhimurium and Enteritidis as recommended previously (Ward et al., 2001).

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References:

Campylobacter Prevalence and Diversity in Antimicrobial Free and Conventionally Reared Market Swine

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Summary: The objectives of this study were to determine the prevalence and antimicrobial resistance of Campylobacter spp. among pigs raised on antimicrobial free (ABF) and those raised conventionally. Bacterial isolation was done on-farm and at slaughter using conventional methods and antimicrobial susceptibility tests were done for 12 antimicrobials using Kirby-Bauer and epielometric test (E-test) methods. All 14 herds were positive for Campylobacter. On-farm prevalence among ABF herds was 71% and 81% among conventional herd. In contrast, the prevalence among carcass swabs was higher among ABF herds than conventional herds with 60% and 29% respectively. There was significant reduction after chilling in all groups (p<0.05). On-farm frequency of antimicrobial resistance was significantly higher among isolates from conventional herds than ABF (p<0.05). In contrast frequency of resistance to five of the seven antimicrobials was higher among carcass swabs of ABF herds than conventional herds.