Use of Pulsed-Field Gel Electrophoresis of Conserved XbaI Fragments for Identification of Swine Salmonella Serotypes

Stephen B. Gaul,1* Stephanie Wedel,1 Matthew M. Erdman,1 D. L. Harris,1,2 Isabel Turney Harris,1 Kathleen E. Ferris,3 and Lorraine Hoffman2

Department of Animal Science,1 Veterinary Diagnostic and Production Animal Medicine,2 Iowa State University, and National Veterinary Services Laboratory,3 Ames, Iowa

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Swine Salmonella isolates (n = 674) from various locations throughout the United States and Canada were analyzed via pulsed-field gel electrophoresis (PFGE) with XbaI. PFGE subtypes were analyzed by cluster analysis and compared to conventional serotyping results. The analysis showed a correlation of serotype to PFGE subtype. In addition, conserved fragments were identified within the restriction patterns that were unique to each serotype. PFGE using XbaI restriction provided a possible alternative method for screening and identifying swine Salmonella serotypes.

Nontyphoidal salmonellae are major causes of food-borne illness in the United States (13). Identifying the serotype of the Salmonella isolates, in combination with molecular subtyping by pulsed-field gel electrophoresis (PFGE), has proven helpful in determining the relatedness of individual cases, indicating an outbreak and its possible source (16). The standard method of serotype identification depends on cell wall O antigens and the flagellar H antigens (6). Since there are currently over 2,400 recognized serotypes, serotyping of unknown isolates has been time-consuming and costly (10, 11).

Attempts have been made to describe the different serotypes by molecular techniques. DNA amplification, such as PCR and amplified length polymorphisms, required a specific set of primers for each serotype (10). Multilocus sequence typing failed to distinguish strains within Salmonella enterica serovar Typhimurium (8). Pulsed-field gel electrophoresis (PFGE) typing, which uses restriction endonucleases to cut the chromosomal DNA into 5 to 20 fragments of various lengths from approximately 10 kb to 900 kb, has proved to be problematic for describing Salmonella serotypes due to perceived variability of results among different laboratories (10).

Of these techniques, PFGE has been the most widely used method to identify and characterize strains of Salmonella within serotypes. Many laboratories have used PFGE to determine strain relatedness, confirm an outbreak of a bacterial disease, and identify the source of a strain or outbreak (1–4). Consequently, PFGE has become a very important tool in epidemiology (14). Garaizar et al. (9) and Ridley et al. (14) used PFGE and computerized gel analysis to identify strains within Salmonella enterica serovar Enteritidis. They concluded that with standardization, PFGE protocols and results were reproducible among different laboratories. They recommended using PFGE and computer databases for epidemiological comparisons. Fakhr et al. (8) concluded that PFGE was superior to multilocus sequence typing in identifying strains of S. enterica serovar Typhimurium. The Centers for Disease Control and Prevention uses PFGE as part of its standard characterization procedures for several key food-borne pathogens and has developed a program called PulseNet to coordinate and collect the PFGE results from various state health departments into a large database (15). Also, Liebana et al. (12) assessed several methods of molecular typing of five selected serovars of Salmonella found in farm animals in the United Kingdom. Their results clearly indicated serotypes of isolates could be identified based on PFGE.

This study was developed to determine if conserved fragments, separated by PFGE, within Salmonella serotypes were suitable to aid in determining the serotype of unknown Salmonella isolates from swine.

MATERIALS AND METHODS

Salmonella isolates were obtained from rectal swabs or pooled pen fecal samples by first using a 24-h preenrichment in 1:9 buffered peptone water (Becton Dickinson, Sparks, MD) incubated at 37°C, followed by a 24-h selective enrichment in 1:99 RV broth (Becton Dickinson) incubated at 42°C and a final streaking for isolated colonies on xylose lysine deoxycholate agar (Becton Dickinson) and incubated overnight at 37°C. Presumptive Salmonella colonies were inoculated into differential media: Kligler’s Media, Simmons Citrate Agar (Becton Dickinson), phenylalanine media, and lysine agar slants. The isolates, which were biochemically identified as Salmonella, were tested with Salmonella O antisera, namely, poly(A) to poly(I) and poly(V), group B factors 1, 4, 5, and 12, and group C1 factor 6 and 7 (Difco), to confirm their identities as Salmonella. Those isolates that tested positive as Salmonella were sent to the National Veterinary Services Laboratory (NVSL) for serotyping.

Isolates of Salmonella enterica subsp. enterica were chosen for inclusion in this study based on the following criteria. The isolates had to be cultured from pigs or swine pens, properly identified as S. enterica through biochemical and serological methods, and have been serotyped by NVSL in Ames, Iowa. This resulted in 650 isolates from 24 different locations from 1998 through 2004 and 11 different serotypes.

In addition, 24 Salmonella isolates were acquired from Iowa State University’s Veterinary Diagnostic Laboratory (ISU VDL). The isolates were from swine samples sent to ISU VDL during 2004.

All isolates were prepared for PFGE by individually suspending the bacterial cells, grown on tryptic soy agar at 37°C, into a cell suspension buffer (100 mM Tris-HCl, 100 mM EDTA, pH 8.0) to a spectrophotometer absorbance of 0.7 ± 0.05 at 612 nm. Proteinase K (20 μl) was added to 400 μl of the suspension along with 400 μl of molten (54°C) 1% Seakem Gold Agar. These were mixed quickly, and approximately 300 μl was dispensed into prepared plug molds. Once solidified,
The 674 isolates, including the isolates from ISU VDL (Table 1), from 12 Salmonella serotypes were separated into 66 different XbaI PFGE subtypes. When the 66 different subtypes were analyzed by cluster analysis, the subtypes were separated into groups of identical serotypes based on their PFGE bands. The groups of individual serotypes were separated from other serotypes at a 70% similarity level (Fig. 1), with 56% similarity over all subtypes. No subtypes of the same serotype clustered together. Closer examination of the patterns (from both ours and published subtypes for DT104 were or nearly were identical to the patterns found in our database. In comparing results from our study to those of the cited papers, the conserved fragments were evident and repeatable, giving support to the view of the clonal origin and spread of DT104 (5). In addition, these results demonstrated that variability in PFGE results among different laboratories should not be a major concern.

In subjecting our PFGE results to cluster analysis, our results were very similar to those described by Liebana et al. (12), in that the cluster analysis grouped identical serotypes together. Closer examination of the patterns (from both ours and Liebana et al. [12]) showed conserved fragments within the PFGE pattern of each serotype, which may be used to assist in identifying the isolate. Also, we compared our results to those of Liebana et al. (12) for XbaI PFGE subtypes of S. enterica serovar Agona. It was found that the same bands mentioned above were conserved. Wonderling et al. (17) used the same analysis program and parameters as those of this study. Their results showed grouping of serotypes by their XbaI PFGE subtypes with cluster analysis.

Our results were in disagreement with the results presented by Kotetishvili et al. (10). Their analysis showed that patterns from the same serotypes were placed in different groups. In observing their PFGE subtypes, two patterns, P3 and P24, were nearly identical with each other but were only 37% similar in their analysis and were placed in different groups. Although

<table>
<thead>
<tr>
<th>Serovar</th>
<th>No. of isolates by location</th>
<th>Total*</th>
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<tbody>
<tr>
<td>Agona</td>
<td>IL 3  IA 24  KY 1  MN 1  MO 1  NE 1  NY 1  NC 1  OK 8  SD 32  WI 16  Canada 16</td>
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<tr>
<td>Brandenburg</td>
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<td>Muenchen</td>
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<td>Putten</td>
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<tr>
<td>Typhimurium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typhimurium (Copenhagen)</td>
<td>6 7 3 81</td>
<td></td>
</tr>
<tr>
<td>Worthington</td>
<td></td>
<td></td>
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</table>

* Total number of isolates, 674.
they used XbaI and the same S. enterica serovar Newport standard as was used in this study, they used a different program to make their analysis and used 3% band tolerances instead of 0.8%, which was used in this study. Of all Salmonella isolates analyzed by PFGE in the study by Kotetishvili et al. (10), only a 22% similarity was found among different groups of isolates. In our study, the similarity was much closer at 56% overall. Analysis of Fig. 1 in Kotetishvili et al. (10) with Bio-

FIG. 1. Cluster analysis of the pulsed-field gel electrophoresis XbaI fragment pattern of Salmonella serovars showing clustering by serovar (Dice similarity coefficient; band tolerance, 0.8%).
Numerics showed that these two patterns, P3 and P24, were indeed the same, and both were identical to our ST18 pattern.

The isolates from ISU VDL showed that PFGE with XbaI was able to predict the serotypes of the isolates. Two isolates of unknown serotype had PFGE subtypes of ST18 and ST21, which were in the database as *S. enterica* serovar Typhimurium (Copenhagen) patterns. These results indicated that when unable to serotype by conventional methods, PFGE would be a possible alternative in serotype determination or may be used to screen isolates for possible serotypes before actual serotyping.

Bands created on a PFGE gel are the product of cut DNA fragments from the genome, whereas serotyping determines the phenotype. Our results indicated that PFGE characterization would be useful as a preliminary screen for the serovar of an isolate of *Salmonella* based on bands conserved within the serotypes’ XbaI PFGE subtypes. If an unserotyped isolate had a PFGE pattern which exactly matches one found in the database, the serotype of the unknown isolate would be identical to the serotype of the matching pattern. If the isolate had a new PFGE pattern, the closest related (>72% similarity) pattern would be of the same serotype. Such PFGE characterization might be especially useful when an isolate cannot be serotyped by conventional methods or when a laboratory does not have access to standard serotyping. Further PFGE characterization, using XbaI restriction, of non-swine isolates would be needed for adding to the database and for serotype screening by PFGE to be applied to *Salmonella* in general.

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**REFERENCES**