Molecular analysis of *Salmonella* serotypes at different stages of commercial turkey processing

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ABSTRACT *Salmonella* isolates were collected from 2 commercial turkey processing plants (A and B) located in different US geographical locations. Isolates recovered at different stages of processing were subjected to 2 genotype techniques [PAGE and denatured gradient gel electrophoresis (DGGE)] to determine their usefulness for *Salmonella* serotyping. Primers used for PCR amplification were to a highly conserved spacer region located between the 16S and 23S rDNA genes. Sampling sites at plant A were 1) postscald, 2) pre-inside-outside bird wash, 3) post-IOBW, and 4) postchill with 30, 44, 36, and 12 *Salmonella* isolates recovered, respectively. Plant B had an additional site and these locations were 1) prescald, 2) postscald, 3) pre-inside-outside bird wash, 4) post-IOBW, and 5) postchill with 16, 54, 24, 35, and 24 *Salmonella* isolates recovered, respectively. In plant A, 4 different *Salmonella* serotypes were identified: Derby, Hadar, Montevideo, and Senftenberg. In plant B, 10 serotypes were identified: Agona, Anatum, Brandenburg, Derby, Hadar, Meleagridis, Montevideo, Reading, Senftenberg, and Typhimurium. *Salmonella* Derby was predominant in plant A (83%), whereas *Salmonella* Typhimurium was the most common serotype recovered in plant B (39%). Genotype analyses of the *Salmonella* serotypes were expressed in dendrograms with comparisons interpreted as percentage similarity coefficients. Both PAGE and DGGE were able to distinguish serotype band patterns. However, DGGE was more discriminating than PAGE. Isolates of the same serotypes were grouped together on the dendrogram of band patterns generated by DGGE. In contrast, PAGE failed to group all like serotypes together on the corresponding dendrogram. The results of the study suggest that genotyping techniques can be very useful in discriminating *Salmonella* serotypes collected from the processing plant environment of commercial poultry production. These molecular techniques may offer more cost-effective means to identify *Salmonella* serotypes from large numbers of isolates and with more immediate results than those currently achieved with conventional typing techniques.

Key words: *Salmonella*, serotype, processing, polyacrylamide gel electrophoresis, denatured gradient gel electrophoresis

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INTRODUCTION

Select serotypes of *Salmonella* collectively represent the predominant bacterial foodborne pathogens affecting humans in the United States. Approximately 10% of food-related illnesses, totaling over 1.4 million cases annually, are associated with foodborne *Salmonella* infection in humans (Mead et al., 1999). Salmonellosis costs the US economy $2.4 billion per year when medical expenses, loss of productivity, and premature death are considered (USDA-ERS, 2005). Poultry meat and eggs are considered to be major vehicles for the transmission of *Salmonella* (Li and Mustapha, 2002; Vadhanasin et al., 2004).

Annually, the Centers for Disease Control (**CDC**) compile a report of the National *Salmonella* Surveillance System in the United States (CDC, 2004). Nonhuman sources of *Salmonella* serotypes provide information that is very useful in epidemiology studies to trace the origin of a known serotype found in human cases. In some instances, the most prevalent serotypes isolated from human and nonhuman sources are the same. In 2004, *Salmonella* Typhimurium and *Salmonella* Heidelberg were reported as the most prevalent serotypes found in nonhuman and human cases (CDC,

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2004). The CDC reported that the serotypes most frequently isolated from chickens were *Salmonella* Heidelberg, *Salmonella* Kentucky, *Salmonella* Senftenberg, *Salmonella* Enteritidis, and *Salmonella* Typhimurium. In turkeys, the following serotypes were identified: *Salmonella* Hadar, *Salmonella* Senftenberg, *Salmonella* Heidelberg, *Salmonella* Muenster, and *Salmonella* Agona (CDC, 2004).

The use of PCR to amplify the spacer region between the 16S and the 23S rDNA is very useful in detecting, identifying, and differentiating bacteria isolates such as *Salmonella* (Kostman et al., 1992; Nastasi and Mammina, 1995; Bakshi et al., 2002). There are significant differences within the spacer region in regard to length and sequence. The wide range of variation among bacteria increases the probability for identification and typing between strains, species, and genera (Gürtler and Stanisich, 1996).

More stringent tests to detect and characterize foodborne pathogens, such as *Salmonella*, must be developed to help safeguard the world's food supply. Recently, a study was conducted to evaluate the abilities of PCR and conventional culture methods to identify *Salmonella* recovered from processed turkey carcasses. The results showed that PCR may improve identification by approximately 43% when compared to conventional methods. The author reported that the highest recovery was observed when PCR and conventional methods were combined (Whyte et al., 2002). Polymerase chain reaction has proven to be very rapid, less labor intensive, very sensitive, reproducible, and more accurate than conventional culture methods. It also presents an excellent diagnostic tool for fast screening and identification of *Salmonella* serotypes in epidemiology studies (Lagatolla et al., 1996; Pritchett et al., 2000; Agarwal et al., 2002).

Molecular-based PCR fingerprinting has become a highly reliable technique for discrimination of genomic DNA in modern research environments. The use of denaturing gradient gel electrophoresis (**DGGE**) and PAGE has been well documented (Muyzer et al., 1993; Ercolini, 2004; Hume et al., 2006). However, the use of DGGE as a diagnostic tool in identifying foodborne pathogens is still in its infancy (Ercolini, 2004). There are marked differences when separating DNA amplicons with regular PAGE and with the PAGE system used in DGGE. Traditional PAGE separates PCR amplicons based on the relative molecular weights of the products. The larger the fragment size, the slower it travels thorough the acrylamide gel. In contrast, DGGE separates PCR products of the same molecular size but with different DNA nucleotide sequences (Ercolini, 2004). Intact DNA is subjected to different concentrations of denaturant in the DGGE acrylamide gel and will eventually separate at different melting domains. When the desired domain is reached, migration of the DNA will stop. As such, DNA with the same relative molecular weight, but different sequences, will migrate to different positions along the gel (Muyzer et al., 1998; Ercolini, 2004).

The addition of a 40- to 50-bp GC clamp to the 5′ end of one of the primers can increase the stability of the double-helix DNA by creating a higher melting domain (Sheffield et al., 1989; Muyzer et al., 1998; Ercolini, 2004). The added stability of the GC clamp allows the newly melted amplicons to separate at their prescribed melting or denaturing levels in the gel, thus stopping the forward migration but remaining connected at the still-intact double-stranded clamp.

The objective of this experiment was to determine the utility of molecular techniques, specifically DGGE or PAGE, to distinguish individual *Salmonella* isolates recovered at various stages of processing in a commercial turkey processing facility. Because few investigations to date have reported upon *Salmonella* serotype isolation within the commercial turkey processing environment, one might presume that current submission charges for conventional serotyping could be prohibiting such investigation. As such, the findings of the current investigation may allow such research to be conducted in a more cost-feasible manner.

MATERIALS AND METHODS

Salmonella Isolation

The *Salmonella* isolates used in this study were recovered during a previous project conducted by our laboratory. The samples were obtained from 2 commercial turkey processing plants (A and B), located in different geographical regions of the United States. The *Salmonella* isolates were recovered at different stages of processing. The sampling sites in plant A were 1) postscald, 2) pre-inside-outside bird wash (**IOBW**), 3) post-IOBW, and 4) postchill, with 30, 44, 36, and 12 recovered isolates per site, respectively. Plant B sampling sites were 1) prescald, 2) postscald, 3) pre-IOBW, 4) post-IOBW, and 5) postchill, with 16, 54, 24, 35, and 24 recovered isolates per site, respectively. The above *Salmonella*-positive isolates were stored on trypticase soy agar (**TSA**) at 4°C, before the start of the current study.

Salmonella isolates in the earlier study were collected from carcasses subjected to whole carcass rinse procedure as previously reported (Cox et al., 1981; USDA-FSIS, 2006). Briefly, 30 mL of collected rinsate was added to 30 mL of buffered peptone water for preenrichment and incubated at 37°C. Subsequently, 0.1 mL of preenrichment broth was used to inoculate 10 mL of Rappaport-Vassiliadis broth and was incubated for 24 h at 42°C. After Rappaport-Vassiliadis enrichment, the samples were enumerated on xylose-lysine-terigitol-4, brilliant green agar (**BGA**), and modified lysine iron agar plates and were then incubated at 37°C for 24 h. All presumptive *Salmonella*-positive isolates were subcultured on triple sugar iron and lysine iron agar slants

for biochemical confirmation of presumptive positives. Subsequently, serological agglutination using polyvalent O and H *Salmonella* antisera was performed on each isolate. *Salmonella*-positive isolates were stored on TSA slants at 4°C, before the start of the current study.

DNA Extraction

An inoculation loop (10 μL) of *Salmonella* from the TSA slants was repeatedly subcultured in tryptic soy broth for 3 d at 37°C. On the third day of subculturing, 1.6 mL of *Salmonella* in tryptic soy broth was placed into 0.4 mL of glycerol and stored at −80°C. In addition, *Salmonella* was streaked onto BGA (containing 25 μg/mL of novobiocin) and grown overnight at 37°C for 18 to 24 h. A colony from each BGA plate was placed in 200 μL of Tris-EDTA buffer (10 m*M* Tris-1 m*M* EDTA, pH 8.0) and placed in boiling water for 15 min. The isolates were chilled and centrifuged at 8,000 × *g* for 10 min. The supernate from each isolate was removed and placed in a clean 1.5-mL centrifuge tube. Deoxyribonucleic acid concentrations were standardized to 15 ng/μL (ND-1000, NanoDrop Technologies, Wilmington, DE) and then stored at −20°C until needed for DNA amplification.

PCR Amplification

PAGE. Polymerase chain reaction primers for PAGE were adapted from the protocol of (Bakshi et al., 2002). Two primers, forward G_1 5′-GAAGTCGTAACA-AGG-3′and reverse L_1 5′-CAAGG CATCCACCGT-3′ (Integrated DNA Technologies, Coralville, IA), were used in the reaction. The mixture final volume was 50 μL and the constituents were as follows: 25 μL of Jumpstart PCR Reaction Mix (Sigma Chemical Company, St Louis, MO), 1 μL of BSA (10 mg/mL), 1.25 μL each of G_1 and L_1 primer (50 ng/μL), 19.5 μL of PCR water (Sigma Chemical Company), and 2 μL of DNA template (15 ng/μL). Amplification of DNA was carried out with a 30-cycle program in a thermocycler (PTC 200, MJ Research Inc., Watertown, MA). The program was as follows: denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 2 min. The final cycle was followed by an additional 7 min at 72°C to complete partial polymerization (Bakshi et al., 2002). Before the start of the first cycle, the mixture was incubated at 94°C for 1 min to increase the final PCR product.

DGGE. Polymerase chain reaction-based DGGE was done according to the method previously reported by (Muyzer et al., 1993) with some modification (Hume et al., 2003). Two primers (50 pmol of each), L_1 5′-CAAGGCATCCACCGT-3′ and G_1 with a GC clamp, 5′-CGCCCGCCGCGC GCGGCGGGCG-GGGCGGGGGCACGGGGGGGAAGTCGTAA-CAAGG-3′ (Integrated DNA Technologies), were mixed with Jumpstart Red-Taq Ready Mix (Sigma Chemical Company) plus 1 μL of BSA (10 mg/mL), 2 μL of DNA template (15 ng/μL), and deionized water was added to make up a final 50-μL volume reaction. Amplification was performed in a thermocycler (PTC 200, MJ Research Inc.) as follows: 1) denaturation at 94.9°C for 2 min; 2) subsequent denaturation at 94°C for 1 min; 3) annealing at 67°C for 45 s, −0.5°C per cycle (touchdown to minimize spurious by-products; Don et al., 1991; Wawer and Muyzer, 1995); 4) extension at 72°C for 2 min; 5) repeat steps 2 to 4 for 17 cycles; 6) denaturation at 94°C for 1 min; 7) annealing at 58°C for 45 s; 8) repeat steps 6 to 7 for 12 cycles; 9) extension at 72°C for 7 min; and 10) held at 4°C for the final stages (Hume et al., 2003).

Gel Electrophoresis

PAGE. A 4-μL aliquot of PCR product was combined with 4 μL of loading buffer (2× loading buffer, Promega, Madison, WI) and 7 μL was loaded per well. The marker well contained 4 μL (0.1 μg/μL) of 100-bp DNA ladder (Ready Load, Invitrogen Life Technologies, Carlsbad, CA) mixed with 4 μL of 2× loading buffer (Promega). Separation of DNA fragments was carried out on a 5% polyacrylamide:bis gel (37.5:1; Bio-Rad Laboratories, Hercules, CA) in 1× Tris-boric acid-EDTA buffer (90 m*M* Tris, 90 m*M* boric acid, and 2 m*M* EDTA, pH 8.4) at room temperature for 17 h at 250 V. After electrophoresis, the gel was stained for 30 min using SYBR Green (1:10,000 dilution, Sigma Chemical Company).

DGGE. The DNA fragments were resolved on a 5% (vol/vol) polyacrylamide:bisacrylamide gel (37.5:1) with a denaturing gradient of 35 to 45% (100% denaturing acrylamide; 7 *M* urea and 40% deionized formamide). Four microliters of PCR product was mixed with an equal volume of 2× loading buffer [0.05% (wt/vol) bromophenol blue, 0.05% (wt/vol) xylene cyanol, and 70% (vol/vol) glycerol] and 7 μL was placed in each sample well (20-well comb). Gel electrophoresis was carried out using 1× Tris-sodium acetate-EDTA buffer (20 m*M* Tris, 10 m*M* sodium acetate, and 0.5 m*M* EDTA, pH 7.4) in a DCode Universal Mutation Detection System (Bio-Rad Laboratories) at 59°C for 17 h at 60 V. After electrophoresis, the gel was stained for 30 min using SYBR Green (1:10,000 dilution, Sigma).

Gel Imaging

The gel images (PAGE and DGGE) were digitalized (Alpha Imager, Alpha Innotech Corporation, San Leandro, CA). The relatedness and dendrogram of fragment patterns were determined with Molecular Analysis Fingerprinting Software (version 1.610, Bio-Rad) based on the Dice similarity coefficient and the unweighted pair group method using arithmetic averages for clustering.

Representative isolates from the different genotypic clusters were selected and sent to USDA-Animal and Plant Health Inspection Service, National Veterinary

Services Laboratory (Ames, IA) for confirmation of serotype. Before serotyping, the isolates were grown on TSA with 5% sheep blood (BBL, Sparks, MD) at 37°C for 18 to 24 h, then a single colony was used to inoculate a TSA slant (Difco, Sparks, MD) and grown for 24 h at 37°C. After serotyping, a representative genotypic isolate from each plant was selected to be compared among all of the different genotypes, thus creating a smaller dendrogram with 14 genotypes, each representing a serotype.

RESULTS

In the present study, *Salmonella* isolates were collected from 2 commercial processing plants (A and B) from 2 geographical locations in the United States. The sampling sites were similar for both plants except that at plant A, due to a scheduling conflict, it was not possible to collect samples at a prescald sampling location.

Plant A

The distribution of the various *Salmonella* serotypes isolated from 4 sampling sites is presented in Table 1. A total of 122 isolates were confirmed to be *Salmonella*-positive. There were 4 sampling sites within the plant: postscald, pre-IOBW, post-IOBW, and postchill, with 30, 44, 36, and 12 *Salmonella* isolates recovered per location, respectively. Of the total 122 *Salmonella* isolates, 22 (18%) representative isolates were selected, from dendrograms generated from preliminary PAGE and DGGE gels (data not shown), to be serotyped. From this subsample, 4 different *Salmonella* serotypes were identified: Derby, Hadar, Montevideo, and Senftenberg.

Approximately 83% of the total isolates recovered in plant A were *Salmonella* Derby, clearly making it the most prevalent serotype recovered within this plant (Table 1). At the postscald location, predominant serotypes recovered were *Salmonella* Derby, *Salmonella* Hadar, and *Salmonella* Senftenberg, with *Salmonella* Derby being recovered at the highest frequency. Postscald recovery generated 25% (30/122) of the total isolates within the plant and all serotypes, with the exception of *Salmonella* Montevideo, recovered in this plant were isolated at this location. More isolates, 36% (44/122), were recovered at pre-IOBW than any other stage of processing. At this location, only 2 serotypes, *Salmonella* Montevideo and *Salmonella* Derby, were found, of which *Salmonella* Derby was the dominant serotype. Fewer *Salmonella* isolates, 30% (36/122), were detected post-IOBW when compared to pre-IOBW. Postchill was the sampling location with the lowest isolation rate (10% or 12/122) as compared to all other locations. *Salmonella* Derby was the only serotype recovered postchill.

Plant B

The results presented in Table 2 represent *Salmonella* recovery frequency by serotype at the different stages of processing sampled in plant B. There were 5 sampling sites in this facility: pre-cald, postscald, pre-IOBW, post-IOBW, and postchill, with 16, 54, 24, 35, and 24 *Salmonella* isolates recovered per site, respectively. Within this facility, a total of 153 isolates were recovered. Forty-nine representative isolates were selected, from dendrograms generated from preliminary PAGE and DGGE gels (data not shown), for serotyping. Ten *Salmonella* serotypes were identified in this plant including, Agona, Anatum, Brandenburg, Derby, Hadar, Meleagridis, Montevideo, Reading, Senftenberg, and Typhimurium.

In contrast to plant A, *Salmonella* Typhimurium (39% or 59/153) was the most frequently isolated serotype in plant B and was the only serotype that was recovered at all sampling sites. At the prescald location, turkeys entered the plant with very low frequency of contamination [10% (16/153)] when compared to other sampling sites. Four *Salmonella* serotypes were isolated prescald, including Derby, Typhimurium, Reading, and Agona, with Derby isolation being at the highest frequency. The highest incidence of *Salmonella* recovery in this plant [35% (54/153)] was at the postscald location, with *Salmonella* Typhimurium and *Salmonella* Derby being the most frequent serotypes isolated. The serotypes *Salmonella* Brandenburg and *Salmonella* Senftenberg were not isolated postscald. Interestingly, there was a shift in serotype recovery at the pre-IOBW sampling site, with *Salmonella* Typhimurium and *Salmo-*

Table 1. *Salmonella* serotypes isolated from plant A turkey carcasses at various processing stages

	Treatment				
Serotype[1]	Postscald	Pre-IOBW[2]	Post-IOBW	Postchill	Total
Derby	13[3] (43.3)[4]	43 (97.7)	33 (91.7)	12 (100)	101 (82.7)
Hadar	9 (30.0)	0 (0.0)	0 (0.0)	0 (0.0)	9 (7.4)
Montevideo	0 (0.0)	1 (2.3)	3 (5.5)	0 (0.0)	4 (3.2)
Senftenberg	8 (26.7)	0 (0.0)	0 (0.0)	0 (0.0)	8 (6.6)
Total	30	44	36	12	122

[1]Conventional antibody serotyping.

[2]IOBW = inside-outside bird wash.

[3]Number of *Salmonella*-positive isolates.

[4]Numbers in parentheses represent the percentage of the total serotypes per column.

Table 2. *Salmonella* serotypes isolated from plant B turkey carcasses at various processing stages

	Treatment					
Serotype[1]	Prescald	Postscald	Pre-IOBW[2]	Post-IOBW	Postchill	Total
Agona	2[3] (12.5)[4]	7 (13.0)	0 (0.0)	0 (0.0)	0 (0.0)	9 (5.9)
Anatum	0 (0.0)	1 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Brandenburg	0 (0.0)	0 (0.0)	12 (50.0)	13 (37.1)	4 (16.7)	29 (18.9)
Derby	7 (43.8)	14 (25.9)	0 (0.0)	0 (0.0)	0 (0.0)	21 (13.7)
Hadar	0 (0.0)	1 (1.9)	2 (8.3)	6 (17.1)	8 (33.3)	17 (1.1)
Meleagridis	0 (0.0)	3 (5.6)	0 (0.0)	0 (0.0)	0 (0.0)	3 (2.0)
Montevideo	0 (0.0)	4 (7.4)	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.6)
Reading	3 (18.8)	4 (7.4)	1 (4.2)	0 (0.0)	0 (0.0)	8 (5.2)
Senftenberg	0 (0.0)	0 (0.0)	1 (4.2)	0 (0.0)	1 (4.2)	2 (2.3)
Typhimurium	4 (25.0)	20 (37.0)	8 (33.0)	16 (45.7)	11 (45.8)	59 (38.6)
Total	16	54	24	35	24	153

[1]Conventional antibody serotyping.

[2]IOBW = inside-outside bird wash.

[3]Number of *Salmonella*-positive isolates.

[4]Numbers in parentheses represent the percentage of the total serotypes per column.

nella Brandenburg being the most common serotypes. Correspondingly, *Salmonella* Reading, *Salmonella* Hadar, and *Salmonella* Senftenberg were isolated at low frequency. *Salmonella* serotype recovery post-IOBW increased by nearly 50% compared to pre-IOBW location. Despite the higher frequency, fewer serotypes were isolated post-IOBW, with recovery of only *Salmonella* Typhimurium and *Salmonella* Brandenburg occurring. At the postchill site, the level of contamination was lower than post-IOBW, and 4 serotypes were isolated: *Salmonella* Typhimurium, *Salmonella* Hadar, *Salmonella* Brandenburg, and *Salmonella* Senftenberg. The most prevalent serotypes postchill were *Salmonella* Typhimurium (46%) and *Salmonella* Hadar (33%).

Cluster Analysis

PAGE. The dendrogram of PAGE genotypes detected (Figure 1) includes representative *Salmonella* serotype isolates found in both plants. Before the generation of the final dendrogram (Figure 1), separate dendrograms for plant A and B were created (data not shown). Plant A sampling resulted in the isolation of 122 distinct *Salmonella* isolates, of which 4 unique PAGE genotypes were identified. Plant B sampling generated the recovery of 153 *Salmonella* isolates, and subsequent analysis revealed 10 PAGE genotypes present. To reduce error, multiple isolates were selected from each genotype for serotype confirmation by the USDA-Animal and Plant Health Inspection Service, National Veterinary Services Laboratory, to ensure validity of the groupings on the PAGE dendrograms.

The results presented in Figure 1 represent the percentage similarity coefficient (**SC**) distribution among *Salmonella* isolates evaluated between plant A and B. The dendrogram was created from representative isolates from each serotype from both plants. A total of 14 representative isolates from 12 genotypes were examined, and overall, all of the genotypes had a 59.9% SC. *Salmonella* Derby (A) and *Salmonella* Reading (B) showed 89.4% SC, whereas the 2 genotypes of *Salmonella* Hadar were grouped together with 91.6% SC. Additionally, genotypes for *Salmonella* Agona, *Salmonella* Brandenburg, and *Salmonella* Montevideo showed 86.3% SC. The banding patterns of *Salmonella* Anatum, *Salmonella* Derby (B), and *Salmonella* Typhimurium (B) displayed 71.7% SC. The 2 genotypes of *Salmonella* Senftenberg had 83.8% SC, whereas *Salmonella* Montevideo was different (71.0%) from all previous serotypes. In addition, *Salmonella* Meleagridis, with a 59.9% SC, was also distinct from all other genotypes. All genotypes displayed similar primary bands between 400 and 600 bp and secondary bands that were useful in discrimination among the different PAGE genotypes.

DGGE. The *Salmonella* isolates previously presented in PAGE comparisons were also used for DGGE analysis (Figure 2). Serotypes (denoted by A or B) were recovered from the different processing plants located in 2 different geographical locations. As previously mentioned, there were a total of 10 serotypes recovered throughout the 2 processing plants. Denatured gradient gel electrophoresis patterns for *Salmonella* Reading and Anatum had 90.1% SC, whereas both Senftenberg isolates were likely identical (98.1% SC). *Salmonella* Agona and *Salmonella* Derby (A) had SC of 90.3%, whereas *Salmonella* Meleagridis was distinct from that group due to an 84.7% SC. The *Salmonella* Hadar genotypes were likely identical (97.6% SC) but were slightly different (92.4% SC) from *Salmonella* Typhimurium. *Salmonella* Derby had 86.8% SC with the other genotypes within the cluster. The 2 *Salmonella* Montevideo genotypes were isolated from 2 different geographical locations (plants A or B) and were genotypically related (89.7% SC). Both *Salmonella* Montevideo isolates, however, were very different from *Salmonella* Brandenburg due to only a 73.0% SC.

DISCUSSION

These findings reveal that *Salmonella* serotypes isolated during commercial turkey processing may vary depending upon geographical location and process-

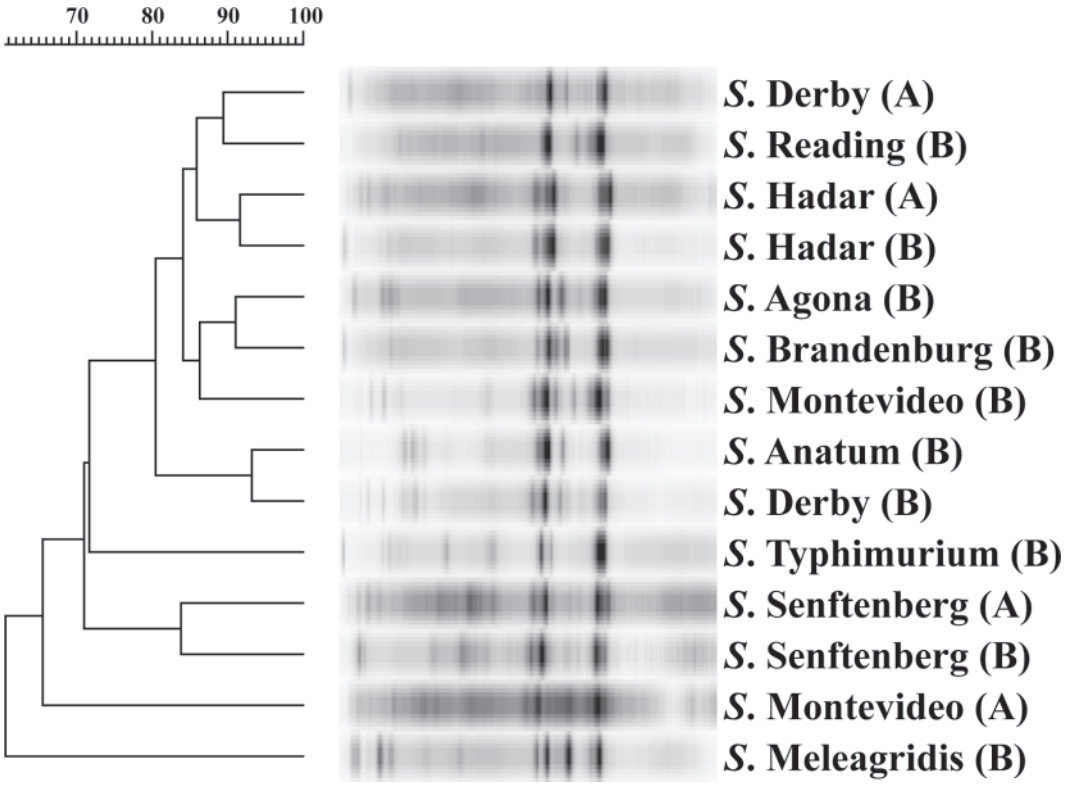

Figure 1. Dendrogram of PAGE band patterns (16 to 23S rDNA) of *Salmonella* serotypes recovered from 2 turkey processing plants (A and B). Percentage similarity coefficient is indicated by the bar above the dendrogram: ≥92% are very related or the same, 85 to 91% are similar, 80 to 84% are somewhat similar, and ≤79% are unrelated (Dunkley et al., 2007).

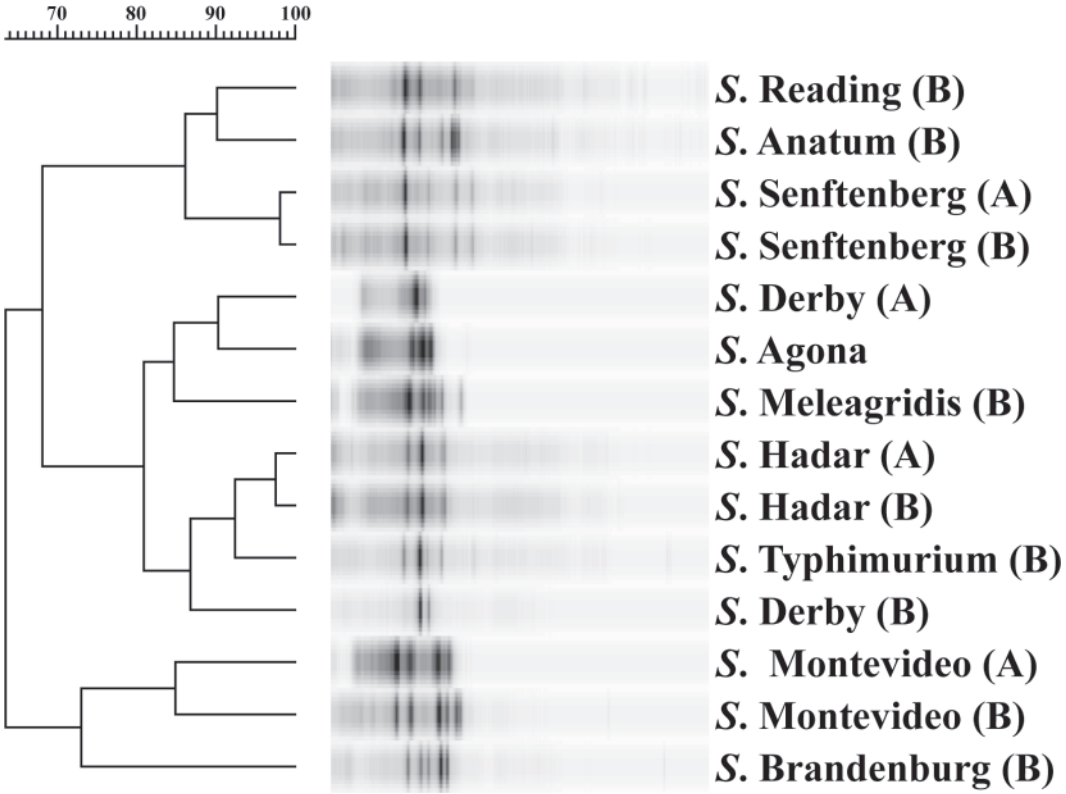

Figure 2. Dendrogram of denaturing gradient gel electrophoresis band patterns (16 to 23S rDNA) of *Salmonella* serotypes recovered from 2 turkey processing plants (A and B). Percentage similarity coefficient is indicated by the bar above the dendrogram: ≥92% are very related or the same, 85 to 91% are similar, 80 to 84% are somewhat similar, and ≤79% are unrelated (Dunkley et al., 2007).

ing facility. A previous study conducted in Malaysia of poultry processing plants demonstrated that different serotypes can be restricted geographically by region. For example, *Salmonella* Blockley was isolated throughout the country, whereas *Salmonella* Enteritidis and *Salmonella* Kentucky were recovered mainly in the central and regional areas, respectively (Rusul et al., 1996). Other factors such as flock population, plant sanitation, age at slaughter, sampling method, season, and management have been shown to influence bacterial recovery in processing plants (Antunes et al., 2003). Only 4 serotypes from the current study were isolated from plant A, whereas plant B sampling resulted in the recovery of 10 distinct *Salmonella* serotypes. *Salmonella* Derby was the most common serotype detected in plant A, whereas in Plant B, the serotype isolated most prevalently was *Salmonella* Typhimurium. This study is somewhat in agreement with several reports that demonstrated that the most common serotypes isolated from poultry were *Salmonella* Enteritidis, *Salmonella* Hadar, and *Salmonella* Typhimurium (Uyttendaele et al., 1998; Leon-Velarde et al., 2004). Most recently, the CDC published their annual summary on *Salmonella* recovery from human and nonhuman sources. According to the report, the 6 serotypes most frequently isolated from human sources were *Salmonella* Typhimurium (19.2%), *Salmonella* Enteritidis (14.1%), *Salmonella* Newport (9.3%), *Salmonella* Javiana (5.0%), *Salmonella* Heidelberg (4.9%), and *Salmonella* Montevideo (2.4%) (CDC, 2004). In our investigation, several *Salmonella* serotypes were present at different stages of processing. The differences in location may be a contributing factor in determining which serotype is more prevalent.

The use of PCR to amplify the variable region between 16S and 23S rDNA has been previously reported (Bakshi et al., 2002). Several researchers are manipulating the 16S and 23S rDNA to discriminate among bacterial species and investigating the usefulness of this characterization in epidemiology studies (Jensen et al., 1993). The PCR-based DGGE molecular fingerprinting technique is more sensitive and reproducible than the standard PAGE; however, it takes much more time to complete. The band patterns from the current study were more distinguishable in DGGE than PAGE. Patterns for corresponding serotypes grouped as very similar in DGGE had higher similarity coefficients than related PAGE groupings. Band pattern comparison uncovered errors in the serotyping of some of the isolates in this study when the fingerprinting profiles were not the same, even though they were reported as the same serotypes. Key features revealing this apparent error in standard serotyping were the distinctive differences in the DGGE genotypic patterns in some comparisons of isolates reported as the same serotype. The anomalous isolates were returned for conventional serotyping to check the credibility of the system. In some cases, the erroneously identified serotypes sent back for reexamination were returned from the diagnostic laboratory with an entirely different and yet still mistaken serotyping. When errors were recognized from antigenic serotyping, analyzing the DNA bands profile was the method of choice to determine the correct serotype. Antigenic traits preventing some isolates from being correctly identified may be indicative of a limited fallibility of the serotyping scheme. However, the potential limited fallibility of the serotyping scheme may be more indicative of antigenic features of some serotypic strains that carry epitopes conveying multiple serotypic identities.

Salmonella serotypes have prominent bands on DGGE gels that are unique to most serotypes and could be classified as primary bands. However, there are secondary bands that provided the main discriminatory tool in distinguishing among the various serotypes (Bakshi et al., 2002). The results from the current study showed that *Salmonella* isolates of the same serotypes, but from different geographical locations in the United States, may differ in DGGE and PAGE band profile. In addition, other factors such as gel alignment and band intensity could affect the genotypic analysis. Evidence of these liabilities was observed among the *Salmonella* serotypes of Derby and Montevideo in Figures 1 and 2. *Salmonella* Senftenberg isolates, although having very similar banding profiles, had only an 83.8% similarity coefficient. The reason for this could be that the banding pattern for one of the isolates was darker than corresponding bands from other isolates, and the analytical program interpreted the patterns as being different. As noted in Figure 2, *Salmonella* Typhimurium appears to be similar to Hadar, but a slightly faded band associated with Typhimurium could have caused the mismatch. It is not always possible to run all isolates on the same gel, or run all isolates at the same time. This restriction could be very problematic with regard to gel alignment in the molecular fingerprinting program. Another point worth mentioning is the necessity for all DNA to be extracted within the same time frame and, when possible, to use the PCR mixture from 1 batch. Such standardization can increase reproducibility by up to 100% (Garaizar et al., 2000).

The use of a molecular fingerprinting technique such as DGGE could be considered as an additional resource to confirm conventional bacterial serotyping based upon serology. Denatured gradient gel electrophoresis is a reliable, accurate, reproducible, and inexpensive technique. In a pandemic scenario, it is necessary to characterize the pathogenic agent quickly, which makes it much easier to identify the best treatment for the affected victims. Therefore, having rapid results would be very economical. Digitizing of images increases the ability to synchronize collaboration between scientists from different laboratories across various regions of the world when working on a possible source and cure for an outbreak. Creating a library or genotypic database of *Salmonella* serotypes would allow for fast identification of unknown serotypes. An added feature of a genotypic approach to *Salmonella* serotyping is reduced cost when numerous isolates are collected during an outbreak and

have to be identified. However, more research of this nature is needed to fully substantiate such claims.

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REFERENCES

Agarwal, A., A. Makker, and S. K. Goel. 2002. Application of the PCR technique for a rapid, specific and sensitive detection of *Salmonella* spp. in foods. Mol. Cell. Probes 16:243–250.

Antunes, P., C. Reu, J. C. Sousa, L. Peixe, and N. Pestana. 2003. Incidence of *Salmonella* from poultry products and their susceptibility to antimicrobial agents. Int. J. Food Microbiol. 82:97–103.

Bakshi, C. S., V. P. Singh, M. Malik, B. Sharma, and R. K. Singh. 2002. Polymerase chain reaction amplification of 16S-23S spacer region for rapid identification of *Salmonella* serovars. Acta Vet. Hung. 50:161–166.

CDC. 2004. *Salmonella* annual summary. http://www.cdc.gov/ncidod/dbmd/phlisdata/salmtab/2004/SalmonellaAnnualSummary%202004.pdf Accessed Jun. 26, 2007.

Cox, N. A., J. E. Thomson, and J. S. Bailey. 1981. Sampling of broiler carcasses for *Salmonella* with low volume water rinse. Poult. Sci. 60:768–770.

Don, R. H., P. T. Cox, B. J. Wainwright, K. Baker, and J. S. Mattick. 1991. 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucleic Acids Res. 19:4008.

Dunkley, K. D., C. S. Dunkley, N. L. Njongmeta, T. R. Callaway, M. E. Hume, L. F. Kubena, D. J. Nisbet, and S. C. Ricke. 2007. Comparison of in vitro fermentation and molecular microbial profiles of high-fiber feed substrates incubated with chicken cecal inocula. Poult. Sci. 86:801–810.

Ercolini, D. 2004. PCR-DGGE fingerprinting: Novel strategies for detection of microbes in food. J. Microbiol. Methods 56:297–314.

Garaizar, J., N. Lopez-Molina, I. Laconcha, D. Lau Baggesen, A. Rementeria, A. Vivanco, A. Audicana, and I. Perales. 2000. Suitability of PCR fingerprinting, infrequent-restriction-site PCR, and pulsed-field gel electrophoresis, combined with computerized gel analysis, in library typing of *Salmonella enterica* serovar Enteritidis. Appl. Environ. Microbiol. 66:5273–5281.

Gürtler, V., and V. A. Stanisich. 1996. New approaches to typing and identification of bacteria using the 16S-23S rDNA spacer region. Microbiology 142:3–16.

Hume, M. E., S. Clemente-Hernandez, and E. O. Oviedo-Rondon. 2006. Effects of feed additives and mixed *Eimeria* species infection on intestinal microbial ecology of broilers. Poult. Sci. 85:2106–2111.

Hume, M. E., L. F. Kubena, T. S. Edrington, C. J. Donskey, R. W. Moore, S. C. Ricke, and D. J. Nisbet. 2003. Poultry digestive microflora biodiversity as indicated by denaturing gradient gel electrophoresis. Poult. Sci. 82:1100–1107.

Jensen, M. A., J. A. Webster, and N. Straus. 1993. Rapid identification of bacteria on the basis of polymerase chain reaction-amplified ribosomal DNA spacer polymorphisms. Appl. Environ. Microbiol. 59:945–952.

Kostman, J. R., T. D. Edlind, J. J. LiPuma, and T. L. Stull. 1992. Molecular epidemiology of *Pseudomonas cepacia* determined by polymerase chain reaction ribotyping. J. Clin. Microbiol. 30:2084–2087.

Lagatolla, C., L. Dolzani, E. Tonin, A. Lavenia, M. Di Michele, T. Tommasini, and C. Monti-Bragadin. 1996. PCR ribotyping for characterizing *Salmonella* isolates of different serotypes. J. Clin. Microbiol. 34:2440–2443.

Leon-Velarde, C. G., H. Y. Cai, C. Larkin, P. Bell-Rogers, R. W. Stevens, and J. A. Odumeru. 2004. Evaluation of methods for the identification of *Salmonella enterica* serotype Typhimurium DT104 from poultry environmental samples. J. Microbiol. Methods 58:79–86.

Li, Y., and A. Mustapha. 2002. Evaluation of four template preparation methods for polymerase chain reaction-based detection of *Salmonella* in ground beef and chicken. Lett. Appl. Microbiol. 35:508–512.

Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. 5:607–625.

Muyzer, G., T. Brinkhoff, U. Nubel, C. Santegoeds, H. Schafer, and C. Wawer. 1998. Denaturing gradient gel electrophoreis (DGGE) in microbial ecology. Pages 1–27 in Molecular Microbial Ecology Manual. A. D. Akkermans, J. D. van Elsas, and F. J. de Bruijn, ed. Kluwer Academic Publishers, the Netherlands.

Muyzer, G., E. C. de Waal, and A. G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59:695–700.

Nastasi, A., and C. Mammina. 1995. Epidemiological evaluation by PCR ribotyping of sporadic and outbreak-associated strains of *Salmonella enterica* serotype Typhimurium. Res. Microbiol. 146:99–106.

Pritchett, L. C., M. E. Konkel, J. M. Gay, and T. E. Besser. 2000. Identification of DT104 and U302 phage types among *Salmonella enterica* serotype Typhimurium isolates by PCR. J. Clin. Microbiol. 38:3484–3488.

Rusul, G., J. Khair, S. Radu, C. T. Cheah, and R. M. Yassin. 1996. Prevalence of *Salmonella* in broilers at retail outlets, processing plants and farms in Malaysia. Int. J. Food Microbiol. 33:183–194.

Sheffield, V. C., D. R. Cox, L. S. Lerman, and R. M. Myers. 1989. Attachment of a 40-base-pair G + C-rich sequence (GC-clamp) to genomic DNA fragments by the polymerase chain reaction results in improved detection of single-base changes. Proc. Natl. Acad. Sci. USA 86:232–236.

USDA-ERS. 2005. Economics of foodborne disease: *Salmonella*. http://www.ers.usda.gov/data/foodborneillness/salmResults.asp?Pathogen=Salmonella&p=1&s=220&y=2008&n=2794374#midForm Accessed Sep. 27, 2007.

USDA-FSIS. 1996. Sample Collection Guidelines and Procedure for Isolation and Identification of *Salmonella* from Raw Meat and Poultry Products. http://www.fsis.usda.gov/OA/fr/rule3.pdf#Appendix%20E Accessed June 2006.

Uyttendaele, M. R., J. M. Debevere, R. M. Lips, and K. D. Neyts. 1998. Prevalence of *Salmonella* in poultry carcasses and their products in Belgium. Int. J. Food Microbiol. 40:1–8.

Vadhanasin, S., A. Bangtrakulnonth, and T. Chidkrau. 2004. Critical control points for monitoring salmonellae reduction in Thai commercial frozen broiler processing. J. Food Prot. 67:1480–1483.

Wawer, C., and G. Muyzer. 1995. Genetic diversity of *Desulfovibrio* spp. in environmental samples analyzed by denaturing gradient gel electrophoresis of [NiFe] hydrogenase gene fragments. Appl. Environ. Microbiol. 61:2203–2210.

Whyte, P., K. Mc Gill, J. D. Collins, and E. Gormley. 2002. The prevalence and PCR detection of *Salmonella* contamination in raw poultry. Vet. Microbiol. 89:53–60.