Research Note

Prevalence, Antibiotic Resistance, and Molecular Characterization of Salmonella Serovars in Retail Meat Products

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ABSTRACT

The prevalence of Salmonella was determined in chicken meat (n = 26), beef (n = 49), and pork (n = 56) collected from wholesale markets, retail stores, and traditional markets in Seoul, South Korea, in 2009. Antibiotic resistance was assessed, and the molecular subtypes of Salmonella isolates were ascertained using an automated repetitive sequence–based PCR (rep-PCR) system (DiversiLab). A total of 18 Salmonella strains were isolated from 17 of 131 samples: 16 strains from each of 16 samples and 2 strains from the same pork sample. The prevalence of Salmonella from the retail meats was 2.0% in beef, 8.9% in pork, and 42.3% in chicken meat. Among 10 different serotypes, Salmonella enterica Panama was recovered from a beef sample, and Salmonella London and Salmonella Montevideo were the predominant serotypes from pork and chicken meat, respectively. The highest antibiotic resistance observed was to erythromycin (100%) followed by streptomycin (22.2%) and tetracycline and chloramphenicol (16.7%). Of the 18 isolates, 5 (27.8%) were resistant to two or more antibiotics, and 1 isolate from chicken meat was resistant to eight antibiotics, including cephalosporins. Differentiation between all of the Salmonella isolates except between Salmonella Montevideo and Salmonella London was successfully performed with the automated rep-PCR system, indicating that it can be added to the toolbox for source tracking of foodborne pathogens associated with outbreaks.

Salmonella enterica serotypes are important foodborne pathogens, and infections caused by Salmonella pose significant public health problems (14, 16). Human salmonellosis is most often caused by the consumption of contaminated foods (18). Raw and processed meat products, including poultry, beef, and pork, are often contaminated with Salmonella (1). Surveillance and monitoring of animal products provide epidemiological data important for preventing foodborne salmonellosis (12).

In recent years, another worldwide health concern has been the occurrence of antibiotic-resistant strains of various pathogenic bacteria (16). The number of Salmonella strains with single- and multidrug resistance has been increasing (14). Some researchers have suggested that the increased use of specific antibiotics in food animals selects for bacteria resistant to antibiotics used in humans, and these resistant bacteria might spread via the food chain to humans and cause human infections, leading to the banning of these antibiotics. Therefore, monitoring the emergence of resistant bacterial strains in foods destined for human consumption is a risk management option that can prevent the development and spread of antimicrobial resistance in microorganisms (16).

To identify sources of infection and pathways of transmission, discrimination of Salmonella isolates below the species level is necessary (26). The traditional method for subspecies typing is serotyping, but this approach has limited utility for epidemiologic analysis of Salmonella transmission because discrimination is poor for closely related isolates (26). Pulse-field gel electrophoresis (PFGE) is a common genotyping method that has been adopted by the Centers for Disease Control and Prevention as the standard for Salmonella genotyping (26). PCR-based genotyping methods are rapid, simple, easy to perform, and more cost effective than PFGE (10). The repetitive sequence–based PCR method (rep-PCR) uses primers for noncoding repetitive sequences and produces DNA fragments that can be separated by electrophoresis (25). This method has been used successfully for more than 10 years to subtype a variety of bacteria, and a commercial semiautomated system, DiversiLab, has been developed (10).

The aims of this 1-year survey were (i) to isolate Salmonella by culture from retail beef, pork, and chicken meat obtained from three types of grocery stores, (ii) to determine the serotype and antimicrobial susceptibility of Salmonella isolates, and (iii) to analyze the genetic relatedness of Salmonella isolates with the rep-PCR method using the DiversiLab system.

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MATERIALS AND METHODS

Sample collection. A total of 131 raw meat samples (beef, pork, and chicken meat) were collected from January to December 2009 from three types of grocery stores in Seoul, South Korea. Stores were stratified by hygiene conditions such as storage temperature, by type of storage (bulk storage or individually wrapped), and by food hygiene practices of the retail food handlers as subjectively determined by the experience of the sample collectors: good (wholesale markets), moderate (retail stores), or poor (traditional markets). Samples included (i) ground beef and brisket of beef \((n = 49)\), (ii) pig forelimb and neck muscle, pork belly, and ground pork \((n = 56)\), and (iii) broiler chicken skin and muscle \((n = 26)\). Samples were individually wrapped, stored in an ice chest, and transported to the laboratory within 1 h for immediate processing.

Salmonella isolation. Salmonella isolation was performed by the culture method according to the Food Code published by the Korea Food and Drug Administration \((11)\). Samples \((25 \text{ g})\) were obtained from each food sample, placed in a sterile bag, diluted 1:9 \((\text{wt/vol})\) in sterile buffered peptone water (BPW; Difco, BD, Sparks, MD), homogenized in a laboratory stomacher for 30 s, and incubated at 37°C for 24 h. One hundred microliters of enriched BPW culture was transferred into 10 ml of Rappaport-Vassiliadis (RV; bioMérieux, Durham, NC) and incubated at 42°C for 24 h for selective enrichment. A loopful of RV enrichment culture was streaked onto xylose lysine deoxycholate agar (Difco, BD), and the plates were incubated overnight at 37°C. A maximum of three presumptive Salmonella colonies from each sample were transferred to triple sugar iron (Difco, BD) and nutrient agar (NA; Difco, BD). The colonies with a positive result by Salmonella-specific real-time PCR were confirmed as Salmonella with API 20E test strips (bioMérieux). Isolates from the same sample that had the same antibiotic susceptibility pattern were regarded as identical.

Real-time PCR. Suspect colonies were resuspended in 1 ml of phosphate-buffered saline in centrifuge tubes for preparation of DNA templates for the real-time PCR assay. Tubes containing bacteria were centrifuged at 16,000 \(\times \text{g}\) for 3 min, and the supernatants were aspirated and discarded. The cell pellets were resuspended in 200 \(\mu\text{l}\) of distilled water. Samples were boiled for 10 min and cooled at room temperature for 2 min. After centrifugation at 16,000 \(\times \text{g}\) for 3 min, the supernatants were collected in new tubes for use as DNA templates. For real-time PCRs, 5 \(\mu\text{l}\) of extracted DNA was added into 20 \(\mu\text{l}\) of PCR mixture, which contained 12.5 \(\mu\text{l}\) of Taqman Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 300 nM forward primer \((5'-\text{CTCACGGGTATCAACATGG-3')}, 900 \text{nM reverse primer} (5'-\text{CTCACGGGTATCAACATGG-3'), and 200 \text{nM probe} (5'-\text{Cy3-CACCGAGGCGGAGACCCGACTTT-BHQ3'). The sequences for the Salmonella-specific oligonucleotide primers and probe were designed to amplify a 94-bp segment of the \(trr\) gene (GenBank accession no. AF 282268) \((15)\). The optimized real-time PCR protocol for Salmonella developed by Primer Express (Applied Biosystems) was performed with an ABI 7500 real-time PCR instrument (Applied Biosystems), specifying two holding periods, one at 50°C for 2 min and another at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. The threshold cycle \((Ct)\), which is the intersection between each fluorescence curve and a threshold line, was calculated with the ABI 7500 software (Applied Biosystems).

Serotyping of Salmonella. The 18 isolates that produced a positive result with the real-time PCR and were identified as Salmonella with the API 20E test were serotyped at the Seoul Metropolitan Government Research Institute of Public Health and Environment of Seoul (J glam-dong, Gwachon, Gyeonggi-Do, South Korea). Serotyping was performed using antisera (Difco, BD) in agglutination tests on the basis of somatic O antigen and phase 1 and phase 2 flagella antigens according to the Kauffmann-White scheme.

Antibiotic susceptibility test. The antibiotic susceptibility of Salmonella isolates was determined by the disk diffusion method according to the recommendation of the NCCLS \((17)\). An antimicrobial susceptibility test disc (Oxoid, Basingstoke, UK) was used for the following antibiotics: 10 \(\mu\text{g}\) of ampicillin, 30 \(\mu\text{g}\) of amikacin, 30 \(\mu\text{g}\) of chloramphenicol, 30 \(\mu\text{g}\) of cephalothin, 5 \(\mu\text{g}\) of ciprofloxacin, 10 \(\mu\text{g}\) of gentamicin, 10 \(\mu\text{g}\) of streptomycin, 25 \(\mu\text{g}\) of sulfamethoxazole-trimethoprim, 30 \(\mu\text{g}\) of tetracycline, 30 \(\mu\text{g}\) of cephaloridine, 30 \(\mu\text{g}\) of amoxicillin–clavulanic acid, 30 \(\mu\text{g}\) of cefepime, 30 \(\mu\text{g}\) of cefoxitin, 30 \(\mu\text{g}\) of cefotaxim, 10 \(\mu\text{g}\) of norfloxacin, and 15 \(\mu\text{g}\) of tetracycline and ciprofloxacin. The results were recorded by measuring the inhibition zones and scored as sensitive, intermediate, and resistant according to the recommendation of the NCCLS. Escherichia coli ATCC 25922 was used as the reference strains.

Rep-PCR DNA fingerprinting with DiversiLab. Salmonella isolates were cultured on NA for 24 h at 37°C. DNA from each isolate was extracted using the UltraClean Microbial DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA) in accordance with the manufacturer’s instructions. Genomic DNA samples were quantified with the NanoDrop 2000 UV spectrophotometer (Thermo Scientific, Wilmington, DE) at 260 nm. All DNA samples and positive and negative controls included in the DiversiLab Salmonella kit were amplified for DNA fingerprinting. For PCR, 2 \(\mu\text{g}\) of genomic DNA (approximately 25 ng/\(\mu\text{l}\)) was added to 23 \(\mu\text{l}\) of PCR mixture, which contained 0.5 \(\mu\text{l}\) of (2.5 U) of AmpliTaq polymerase (Applied Biosystems), 2.5 \(\mu\text{l}\) of 10 × GeneAMP PCR buffer I (Applied Biosystems), 2 \(\mu\text{l}\) of kit-supplied primer mix, and 18 \(\mu\text{l}\) of the kit-supplied rep-PCR master mix (MM1) in the DiversiLab kit. The thermal cycling parameters were as follows: initial denaturation at 94°C for 2 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 70°C for 90 s, and a final extension at 70°C for 3 min. The rep-PCR products were separated and detected with a microfluidics chip and a model 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). There were analyzed simultaneously on a chip, and internal DNA standards of known sizes were added to each well to allow for normalization and efficient chip-to-chip comparisons. For analysis, all samples were analyzed and compared with a Pearson correlation analysis using the Web-based DiversiLab software (version 2.1.66) to assess band position and intensity, and the distance matrices and the unweighted pair group method with arithmetic mean were determined to create dendograms \((9)\).

Statistical analysis. The prevalence of Salmonella from food obtained from the three types of grocery stores was analyzed with Fisher’s exact test using InStat software (version 3.05, GraphPad, San Diego, CA).

RESULTS AND DISCUSSION

Prevalence of Salmonella. A total of 131 meat products were obtained from three types of grocery stores in South Korea between January and December 2009. Salmonella was isolated from 17 samples: 2.0% (1 of 49) of
beef samples, 8.9% (5 of 56) of pork samples, and 42.3% (11 of 26) of chicken meat samples (Table 1). The prevalence of Salmonella in beef and pork samples in this study was in close agreement with that found in the same types of meat in developed countries: 1.9% in beef and 3.3% in pork in the greater Washington, DC, area (28) and 0% in beef and 9.9% in pork in Ireland (8). However, the prevalence was lower than that reported in studies conducted in other developing countries: 15.1% in ground beef and 17.3% in ground pork in Mexico (16) and 65% in beef and 64% in pork in Vietnam (24). The prevalence of Salmonella from retail chicken meat obtained in this study was higher than that in other meat products but similar to that previously reported: 36.0% in Korea (6), 36.5% in Belgium (23), 35.5 to 47.7% in Austria (21), and 53.3% in Vietnam (24). The prevalence of Salmonella in previous studies was associated with differences in hygiene and sanitation levels and in detection methods used in each study.

We compared the prevalence of Salmonella in three types of markets with different hygiene conditions (good, moderate, or poor hygiene): wholesale markets, retail stores, and traditional markets. The traditional markets had the highest prevalence of Salmonella (17.1%, 7 of 41 samples) compared with that in the wholesale markets (10%, 4 of 40 samples) and retail stores (11.8%, 6 of 51 samples) (Table 1), but differences were not significant (P > 0.05). These results suggest that the prevalence of Salmonella may be associated with the hygiene conditions of the facility.

**Distribution of Salmonella serotypes.** Eighteen Salmonella strains were isolated from 17 of 131 samples: 16 strains from each of 16 samples and two different serotypes from the same pork sample (Table 2). Ten Salmonella serovars were identified from beef, pork, and chicken meat samples. One beef sample was positive for Salmonella Panama (Table 2). Six Salmonella isolates were identified from five pork samples. Three pork samples (60%) were contaminated with Salmonella London, one sample (20%) with Salmonella Rissen, and one sample (20%) with both Salmonella Enteritidis and Salmonella Typhimurium. These results are similar to those of Lim et al. (13), who reported that Salmonella Typhimurium (38.9%) and Salmonella Rissen (25.3%) were the most common in healthy pigs, while Salmonella Typhimurium (89.7%) was common in diarrheic pigs in Korea.

Salmonella isolates from 11 chicken meat samples belonged to six serovars: Salmonella Montevideo was isolated from 4 samples (36.4%), Salmonella Dessau was isolated from 3 samples (27.3%), and Salmonella Agona, Salmonella Enteritidis, Salmonella Lome, and Salmonella Weltevreden were each isolated from 1 sample (9.1%). In a previous prevalence study in Korea, Salmonella Enteritidis and Salmonella Typhimurium were the most common Salmonella serotypes isolated from chicken meat (5, 6). Cheong et al. (5) also reported that Salmonella Montevideo, the most common serotype in our study, was isolated from 6% of broiler chickens in Korea. However, in the present study, various serotypes were isolated from chicken, only one of which was Salmonella Enteritidis.

**Antimicrobial resistance profiles.** The antibiotic susceptibility patterns of Salmonella isolates are shown in Table 2. All Salmonella isolates from retail beef, pork, and chicken meat were resistant to erythromycin. In many previous studies, Salmonella isolates from foods were highly resistant to erythromycin (3, 22, 27). Cardoso et al. (3) reported that all Salmonella Enteritidis strains from broiler carcasses in Brazil were resistant to erythromycin and tetracycline.

For the remaining 17 antimicrobial agents tested, the highest resistance rate observed was to streptomycin (22.2%) followed by tetracycline and chloramphenicol (16.7%) and ciprofloxacin and ampicillin (11.1%). In many previous studies, many Salmonella isolates have been resistant to streptomycin, ampicillin, and tetracycline (5–7, 16, 20, 28). Tetracycline, streptomycin, nitrofurantoin, and ampicillin have been commonly used in the Korean broiler industry (6). The use of antibiotics in the animal husbandry may lead to antibiotic resistance of pathogens in animal products. The results of the present study are in agreement with antibiotic resistance profiles of Salmonella isolates from patients with diarrhea: streptomycin (100% of samples) and erythromycin (90.6% of samples) in Busan, South Korea (4), and tetracycline (32.7% of samples), streptomycin (28.0% of samples), and ampicillin (12.4% of samples) in Seoul, South Korea (19).

In the present study, 5 (27.8%) of 18 isolates were resistant to two or more antibiotics. Among these five isolates, three were resistant to streptomycin, chloramphenicol, and erythromycin. A Salmonella Lome isolate from chicken meat from the traditional market was resistant to eight antibiotics, including cephalosporins (cephalothin, cefazolin, and cefotaxim). None of the Salmonella strains isolated in this study were resistant to the β-lactam–β-lactamase inhibitor combination (amoxicillin–clavulanic acid), carbapenem (imipenem), and quinolones (ciproflox-
acinar, norfloxacin, and enrofloxacin). Resistance to quinolones and cephalosporins is of primary medical concern in the treatment of Salmonella infections (28). In previous studies in Korea (5–7), no Salmonella isolates from foods were resistant to quinolones and cephalosporins, but in the present study one isolate from chicken meat was resistant to three of the five cephalosporins. These results suggest that regulations prohibiting inappropriate use of antibiotics without a veterinarian’s prescription should be required in the animal industry in Korea.

Molecular characterization by rep-PCR fingerprinting. To our knowledge, this study is the first in which rep-PCR was used as an alternative to PFGE for genotyping of Salmonella isolated from naturally contaminated foods from various sources. The dendrogram generated from the rep-PCR patterns and computer-generated virtual gel images are summarized in Figure 1. We compared the rep-PCR patterns of Salmonella isolates of different serotypes, antibiotic resistance profiles, and meat sources.

Salmonella Enteritidis from pork had greater than 95% similarity with Salmonella Lome in the same serogroup (D1) but not with Salmonella Panama, which had 64.1% similarity. In serogroups B, C1, and E1, similarity of rep-PCR pattern was not observed between serotypes in the same serogroup: Salmonella Typhimurium and Salmonella Agona of serogroup B, Salmonella Rissen and Salmonella Montevideo of serogroup C1, and Salmonella Weltevreden and Salmonella London of serogroup E1. These results indicate that similarity in rep-PCR pattern tends to be not related to Salmonella serogroup.

The rep-PCR assay successfully differentiated Salmonella Dessau and Salmonella Enteritidis isolates, with less than 90% similarity. However, some serotypes of Salmonella may not be distinguishable by rep-PCR. Salmonella isolates of the same serotype and antibiotic resistance pattern, Salmonella Montevideo and Salmonella London, had greater than 93% similarity even though these strains were isolated from meats from different markets (Table 2). These results are in agreement with those of Wise et al. (26). These authors created a library of rep-PCR patterns from 14 Salmonella serotypes and successfully determined the serotype of unknown Salmonella isolates by comparison with the library (26). Therefore, rep-PCR has a limited ability to discriminate serotypes of Salmonella from different sources. In many studies, the traditional genotyping method, PFGE analysis, also has produced the same banding patterns for same serotypes from different sources (2). Therefore, a combination of both genotyping methods, rep-PCR and PFGE, could more effectively distinguish Salmonella isolates of the same serotype.

In other studies in which rep-PCR and PFGE analyses have been compared, the rep-PCR method provided highly reproducible fragmentation patterns and greater discriminative power for closely related Salmonella isolates (10, 25). Kilic et al. (10) reported that PFGE and rep-PCR results were concordant for Salmonella Enteritidis isolates from patients and food samples associated with a foodborne outbreak. The DiversiLab system for rep-PCR was technically simple. It allowed completion of analysis of 12 samples in approximately 4 h, compared with 3 days for PFGE, and Web-based software is available for easy comparison of samples between international laboratories.

In conclusion, the prevalence of Salmonella was the highest in chicken meat samples, and one isolate from meat from a traditional market was resistant to eight antibiotics, including cephalosporins. High resistance rates were

### Table 2. Serovars and antimicrobial resistance patterns of Salmonella isolates identified in this study

<table>
<thead>
<tr>
<th>Sample</th>
<th>Market type</th>
<th>Isolate ID</th>
<th>Salmonella serovar</th>
<th>Serogroup</th>
<th>Resistance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef</td>
<td>Wholesale</td>
<td>Beef-1</td>
<td>Panama</td>
<td>D1</td>
<td>E</td>
</tr>
<tr>
<td>Pork</td>
<td>Wholesale</td>
<td>Pork-1</td>
<td>Enteritidis</td>
<td>D1</td>
<td>AMP/S/C/E</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pork-2</td>
<td>Typhimurium</td>
<td>B</td>
<td>CN/S/C/E/TE</td>
</tr>
<tr>
<td>Traditional</td>
<td></td>
<td>Pork-3</td>
<td>Rissen</td>
<td>C1</td>
<td>S/C/E/TE</td>
</tr>
<tr>
<td>Retail</td>
<td></td>
<td>Pork-4</td>
<td>London</td>
<td>E1</td>
<td>E</td>
</tr>
<tr>
<td>Chicken meat</td>
<td>Wholesale</td>
<td>Chicken-1</td>
<td>Agona</td>
<td>B</td>
<td>E</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chicken-2</td>
<td>Dessau</td>
<td>E4</td>
<td>CN/E</td>
</tr>
<tr>
<td>Traditional</td>
<td></td>
<td>Chicken-3</td>
<td>Montevideo</td>
<td>C1</td>
<td>E</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chicken-4</td>
<td>Montevideo</td>
<td>C1</td>
<td>E</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chicken-5</td>
<td>Dessau</td>
<td>E4</td>
<td>E</td>
</tr>
<tr>
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<td></td>
<td>Chicken-6</td>
<td>Dessau</td>
<td>E4</td>
<td>E</td>
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<tr>
<td></td>
<td></td>
<td>Chicken-7</td>
<td>Lome (Vi+)</td>
<td>D1</td>
<td>AMP/KZ/KF/CN/S/CTX/E/TE</td>
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<td>Enteritidis</td>
<td>D1</td>
<td>E</td>
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<tr>
<td></td>
<td></td>
<td>Chicken-9</td>
<td>Montevideo</td>
<td>C1</td>
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<td>C1</td>
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<tr>
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<td></td>
<td>Chicken-11</td>
<td>Weltevreden</td>
<td>E1</td>
<td>E</td>
</tr>
</tbody>
</table>

*AMP, ampicillin; C, chloramphenicol; CN, gentamicin; CTX, cefotaxim; E, erythromycin; KF, cephalothin; KZ, cefazolin; S, streptomycin; TE, tetracycline.

*Salmonella* isolates were recovered from the same sample.
observed for erythromycin (100% of samples) and streptomycin (22.22% of samples). Therefore, meat products contaminated by Salmonella could present a serious risk for human health. Differentiation among all Salmonella isolates except Salmonella Montevideo and Salmonella London was successful with the automated rep-PCR system DiversiLab. This method could be added to the toolbox in combination with PFGE for source tracking of foodborne pathogens in outbreaks and for confirming cross-contamination from the working environment.

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REFERENCES


