Prevalence, characterization, and antimicrobial susceptibility of *Salmonella* Gallinarum isolated from eggs produced in conventional or organic farms in South Korea

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**ABSTRACT** To determine the prevalence of *Salmonella* serotype Enteritidis in eggs in South Korea, we conducted a microbiological survey of commercially available eggs produced in conventional or organic farms during the period from 2010 to 2012. The contents of 7,000 raw shell eggs (6,000 of conventional and 1,000 of organic origin) were examined to evaluate the extent and type of *Salmonella* Enteritidis contamination. A total of 26 salmonellae (7.4% of all pooled samples) were isolated from 350 homogenized pools, each containing the contents from 20 eggs. An unexpected and particularly surprising finding was that all the *Salmonella* isolates were serotyped as *Salmonella* Gallinarum. *Salmonella* Gallinarum was more common in eggs from organic farms: 10 of 50 egg pools (20.0%) from organic and 16 of 300 egg pools (5.3%) from conventional farms tested positive for *Salmonella* Gallinarum. However, organic and conventional isolates showed similar antimicrobial susceptibilities. All the isolates and a vaccine strain, SG 9R, which has been widely used in South Korea, were further characterized using the automated repetitive sequence-based PCR (rep-PCR) system, DiversiLab, to ascertain the molecular subtypes and to identify differences from the vaccine strain. The rep-PCR identified 2 distinct clusters among the 26 *Salmonella* Gallinarum isolates with a greater than 96% similarity index. These were clearly differentiated from the vaccine strain, SG 9R, with which there was a less than 86% similarity index. We found there was low genetic heterogeneity among isolates within each cluster and were able to distinguish wild type strains from the live vaccine strain (SG 9R) using the DiversiLab system.

**Key words:** shell egg, *Salmonella* Gallinarum, SG 9R, vaccination, automated repetitive sequence-based PCR

2013 Poultry Science 92:2789–2797
http://dx.doi.org/10.3382/ps.2013-03175

**INTRODUCTION**

Foodborne diseases may be one of the most significant public health problems, considering the large number of cases and the associated economic costs (Motarjemi and Käferstein, 1997). Industrialization, mass production of food, decreasing trade barriers, and human migration have disseminated and increased the incidence and severity of foodborne diseases worldwide (Gomez et al., 1997; Käferstein et al., 1997; Todd, 1997).

Salmonellae are among the most common pathogens associated with foodborne diseases (Käferstein, 2003; Mead et al., 1999). Most human infections are acquired by eating foods of animal origin such as beef, pork, poultry, eggs, or dairy products that may be contaminated with *Salmonella* (Gebreyes et al., 2000; Rajashekara et al., 2000).

Contaminated poultry-derived products, particularly chicken eggs, are important vehicles of *Salmonella* infections, especially when the bacterium is in the egg contents. This issue was recently highlighted in the *Salmonella* outbreak in shell eggs that occurred in the United States in May 2010 (US Food and Drug Administration, 2010). It was caused by *Salmonella enterica* serovar Enteritidis (*Salmonella* Enteritidis) that was traced to contaminated shell eggs from Iowa (Kuehn, 2010).

Microbiological surveys have played an important role in understanding the extent and pattern of *Salmonella* contamination in eggs. In Korea, there has been little research on this issue in egg marketing channels.
Consequently, there is minimal background information or data on the prevalence of Salmonella serovars in the contents of shell eggs. In addition, national systems to monitor egg quality are insufficient, and few guidelines have been prescribed for the storage of eggs in retail markets.

The emergence of resistant bacterial strains is another worldwide health concern. The results of monitoring Salmonella strains with single drug and multidrug resistance can be used to establish a selection guide for antimicrobial therapy (Miranda et al., 2009). Studies on the prevalence of Salmonellae and characterization of their antimicrobial resistance spectra have largely been restricted to isolates from clinical or veterinary sources or both.

The present study was undertaken primarily to assess the level of Salmonella contamination in the contents of shell eggs, with special reference to Salmonella Enteritidis. The major study objectives were to determine the prevalence and antibiotic resistance pattern of Salmonella serovars isolated from the contents of shell eggs produced at organic or conventional farms in South Korea during the period from 2010 to 2012. Molecular characterization was also carried out using the DiversiLab system to evaluate the genetic relatedness of the Salmonella isolates and a live vaccine strain (SG 9R) that has been widely used in layer chicken farms in South Korea.

MATERIALS AND METHODS

Sample Collection

From November 2010 to March 2012, a total of 7,000 shell eggs (350 pooled samples of 20 eggs each) were randomly collected, 1,000 from an organic farm and 6,000 from 3 conventional farms in South Korea. The pooling criterion was based on the FDA Bacteriological Analytical Manual (BAM; US Food and Drug Administration, 2012). All eggs were transported to the laboratory within 2 d of being laid.

Preparation of Homogenized Egg Pools

To isolate Salmonella from egg contents, shell eggs were prepared according to the BAM protocol (US Food and Drug Administration, 2012). Any visibly cracked eggs were discarded, and any adherent materials on the shell surface were removed. To disinfect the shells, eggs were soaked in a mixture of 3 parts 70% alcohol to 1 part iodine/potassium iodide solution for at least 10 s and then air-dried (US Food and Drug Administration, 2012). Eggs were cracked aseptically and their contents combined in pools of 20 eggs for a total of 350 bulk pools, 50 pools from organic and 300 from conventional farms. The contents of each bulk pool were mixed manually until the yolks were completely mixed with the albumen (Seo et al., 2003; Valentin-Bon et al., 2003). The homogenized pools were then incubated for 96 h at room temperature (20–24°C).

Salmonella Isolation

Salmonella isolation from egg contents was performed by the culture method described in the FDA BAM (US Food and Drug Administration, 2012). After incubation for 96 h at room temperature, 25-mL samples of the pooled and homogenized eggs were mixed with 225 mL of sterile trypticase soy broth (TSB; Difco, Detroit, MI) supplemented with ferrous sulfate (35 mg of ferrous sulfate per 1,000 mL of TSB). The egg and enrichment broth mixtures were incubated at 35°C for 24 h. One milliliter of each broth culture was added to 10 mL of Muller-Kauffmann tetrathionate broth with novobiocin (bioMérieux, Marcy l’Etoile, France) and incubated for 24 h at 37°C. Preenriched TSB culture (100 µL) was transferred into 10 mL of Rappaport-Vassiliadis Soya (bioMérieux) and incubated for 24 h at 42°C for selective enrichment. A loopful of each Rappaport-Vassiliadis Soya and Muller-Kauffmann tetrathionate broth with novobiocin culture was streaked onto bismuth sulfite, brilliant green, and xylose lysine desoxycholate agar plates (Oxoid, Hampshire, UK). The plates were incubated for 24 h at 37°C. Bismuth sulfite agar plates were incubated for an additional 24 h (48 h total). Presumptive Salmonella colonies were subjected to further standard metabolic and biochemical tests, and those with a positive result by Salmonella-specific real-time PCR were confirmed as Salmonella using the VITEK system (bioMérieux).

Real-Time PCR

To confirm presumptive Salmonella, real-time PCR reactions were performed using an ABI 7500 real-time PCR (Applied Biosystems, Foster City, CA). Primers and probe sequences and cycling conditions specific for Salmonella were done according to Malorny et al. (2004). The sequences for the Salmonella-specific oligonucleotide primers and probe were designed to amplify a 94-bp segment of the ttr gene (GenBank accession no: AF 282268). Primer and probe sequences were as follows: forward primer (5'-CTC ACC AGG AGA TTA CAA CAT GG-3'), reverse primer (5'-AGC TCA GAC CAA AAG TGA CCA TC-3'), and probe (5'-Cy3-CAC CGA CGG CGA GAC CGA CT'T-BHQ3-3').

For the preparation of DNA templates, colonies were suspended in 1 mL of PBS and centrifuged at 16,000 × g for 3 min at 4°C. After the supernatants were aspirated and discarded, the cell pellets were resuspended in 200 µL of PrepMan Ultra reagent (Applied Biosystems) and boiled at 100°C for 10 min. The boiled cell suspensions were cooled at room temperature for 2 min, centrifuged at 16,000 × g for 3 min, and the supernatants collected in new tubes for use as DNA templates. The supernatants (5 µL of extracted DNA) were trans-
fered to 20 μL of PCR reaction mixture consisting of Taqman Universal PCR Master Mix (12.5 μL; Applied Biosystems), 300 nM forward primer (2.5 μL), 900 nM reverse primer (2.5 μL), and 200 nM probe (2.5 μL). The microwell plates were sealed and placed in an ABI 7500 real-time PCR instrument (Applied Biosystems).

An optimized real-time PCR protocol for Salmonella developed by ABI 7500 was performed. The reaction was run at 50°C for 2 min, then 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 using the ABI 7500 software (Applied Biosystems).

Serotyping of Salmonella

Serotyping of Salmonella isolates was performed using commercially available antisera. Agglutination test was conducted by using Salmonella O antiserum Group D1 (Difco) and Salmonella H antiserum single factor m (Difco). The agglutination properties were identified based on antigenic classification according to the Kauffmann-White scheme.

Identification of Salmonella Gallinarum by Colony PCR Assay

The validation of serotyping was conducted with colony PCR targeting the Salmonella Gallinarum specific gene (rfbS gene). One colony subcultured on tryptic soy agar (Oxoid) was removed and genomic DNA was extracted by the boiling method. Primer sequences and cycling conditions specific for rfbS gene were done according to Jeon et al. (2007). The final PCR reaction mixture (20 μL) consisted of Maxime PCR PreMix (iTRON Biotechnology, Sungnam, Korea), 50 ng of template DNA, and 20 pmol of each primer.

Antibiotic Susceptibility Test

All the Salmonella isolates were investigated for their antibiotic susceptibility patterns. Colonies were picked and suspended in sterile saline, adjusted to 0.5 McFarland standard, and inoculated on Mueller-Hinton agar (Oxoid). Antimicrobial resistance was assessed by the disc diffusion method on Mueller-Hinton agar (Difco; CLSI, 2012).

A total of 17 antimicrobial agents currently in use in the veterinary and medical fields were tested. All the isolates were screened for antimicrobial susceptibility using test discs (Oxoid) containing the following antibiotics: 10 μg of ampicillin, 30 μg of amikacin, 30 μg of chloramphenicol, 30 μg of cephalothin, 5 μg of ciprofloxacin, 10 μg of gentamicin, 10 μg of streptomycin, 25 μg of sulfamethoxazole-trimethoprim, 30 μg of tetracycline, 30 μg of cefazolin, 30 μg of amoxicillin–clavulanic acid, 30 μg of cefoxitin, 30 μg of cefotaxim, 5 μg of enrofloxacin, 10 μg of norfloxacin, 10 μg of imipenem, and 15 μg of erythromycin. The inhibition zones were measured and the results interpreted as sensitive, intermediate, or resistant (CLSI, 2012). In all the cases, Escherichia coli ATCC 25922 was used as the reference strain.

Strain Typing Using the DiversiLab Automated Repetitive Sequence-Based PCR System

Salmonella isolates, a vaccine strain, SG 9R (Nobilis, Intervet Korea, Seoul, Korea), and Salmonella Enteritidis pork-1 strain isolated from pork meat in 2011 were cultured on nutrient agar for 24 h at 37°C. The DNA from each isolate and the vaccine strain was extracted using the UltraClean Microbial DNA Isolation Kit (MO BIO Laboratories Inc., Solana Beach, CA) according to the manufacturer’s instructions. The concentration of genomic DNA was estimated using a NanoDrop 2000 UV Spectrophotometer (Thermo Scientific Inc., Wilmington, DE). The DNA concentration for each sample was adjusted to 25 to 50 ng/μL by measuring the absorbance at 260 nm with the NanoDrop 2000 UV spectrophotometer. All the DNA samples were amplified using the DiversiLab Salmonella Kit for DNA fingerprinting (bioMérieux), following the manufacturer’s instructions. Briefly, 2 μL of genomic DNA (concentration approximately 25 ng/μL), 0.5 μL (or 2.5 U) of AmpliTaq polymerase (Applied Biosystems), 2 μL of kit-supplied primer mix, and 2.5 μL of 10 × Gene AMP PCR Buffer I (Applied Biosystems) were added to 18 μL of the kit-supplied automated repetitive sequence-based (rep-PCR) master mix (MM1) for a total of 25 μL/PCR reaction mixture. Thermal cycling parameters were as follows: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, extension at 70°C for 90 s, and a final extension at 70°C for 3 min.

The rep-PCR products were separated with the micro-fluidic chips of the DiversiLab System (bioMérieux) and detected using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). All the samples were analyzed and compared using the web-based DiversiLab software (version 2.1.66) to assess band position, intensity, and the distance matrices. The resulting rep-PCR patterns were viewed as a dendrogram constructed from a similarity matrix, and as a virtual gel image of the fingerprint for each DNA sample.

Statistical Analysis

The prevalence of Salmonella and resistant profiles were compared between 2 sources of shell eggs. Contingency tables were made for comparison of categorical variables, and were analyzed by Fisher’s exact test using InStat version 3.05 (GraphPad Software, San Diego, CA). The P-values were derived using Fisher’s
exact test and $P$-values < 0.05 were considered statistically significant.

### RESULTS AND DISCUSSION

**Prevalence and Serotyping of Salmonella**

*Salmonella* were isolated from 26 (7.4%) of the 350 pooled samples: 10 of 50 (20%) from organic egg pools and 16 of 300 (5.4%) from conventional egg pools (Table 1). This represents a statistically significant difference between organic and nonorganic shell eggs ($P < 0.05$).

The fact that *Salmonella* were isolated more frequently from organic eggs (20%) than conventional eggs (5.4%) suggests that the extent of *Salmonella* contamination may be affected by whether or not antibiotics are used in egg production. All *Salmonella* isolates were serotyped as *Salmonella* Gallinarum (*Salmonella Gallinarum*), implying that this serotype is generally distributed in South Korean egg farms. All isolated *Salmonella* Gallinarum strains were confirmed by using colony PCR assay. This is interesting as it suggests that SG 9R, a live fowl-typhoid vaccine in use in South Korea since 2001, might be ineffective in preventing fowl typhoid or in limiting transmission of *Salmonella* Gallinarum in eggs (Smith, 1956). There have been many previous reports stating that the SG 9R vaccine does not limit transmission of *Salmonella* Gallinarum as it could be isolated from the eggs of birds vaccinated subcutaneously and later challenged with the bacterium (Silva et al., 1981a,b). Previous studies also indicated that adult birds vaccinated with a rough strain of *Salmonella* Gallinarum may be capable of vertically transmitting both the SG 9R vaccine strain and pathogenic strains (Silva et al., 1981a,b). The potential for egg transmission of the SG 9R strain following vaccination and of a pathogenic strain following challenge against vaccinated birds was indicated by detecting ovarian infections with each strain and by the isolation of the pathogenic strain from eggs (Silva et al., 1981a,b).

The 9R vaccine strains may still give rise to systemic disease and bacterial persistence for several weeks in young chickens, even though they are highly attenuated compared with the parental *Salmonella* Gallinarum strain (Smith, 1969; Feberwee et al., 2001; Wigley et al., 2005; Lee et al., 2007). It is therefore not easy for veterinary clinicians using routine biochemical tests to determine whether a chicken was infected by pathogenic field bacteria or the 9R vaccine strain (Lee et al., 2007). To differentiate the isolated *Salmonella* from the vaccine strain, we used further subtyping methods, including antimicrobial susceptibility testing and rep-PCR fingerprinting.

### Antimicrobial Resistance Profile

The SG 9R was resistant to erythromycin and streptomycin and susceptible to all 17 of the other antibiotics. Seven (11.5%) of the *Salmonella* Gallinarum isolates were resistant to erythromycin and susceptible to all the other antibiotics. The remaining 23 isolates (88.5%) were resistant to erythromycin and intermediate resistant to streptomycin (Table 2). None of the strains we isolated were fully resistant to streptomycin, distinguishing them from SG 9R and indicating that antibiotic resistance profile may be used as a biomarker to discriminate wild type *Salmonella* Gallinarum strains from the vaccine strain, SG 9R.

The erythromycin resistance of *Salmonella* Gallinarum observed in the current study is in agreement with other studies (Giurov, 1980; M. R. Akter, 2007; Shapouri et al., 2009). We also detected intermediate resistance to streptomycin in 23 isolates (88.5%) in the current study; resistance to streptomycin was less frequent in the earlier reports on *Salmonella* Gallinarum (Rzedzicki and Glinski, 1975; Giurov, 1986). These results suggest that the reduced susceptibility to streptomycin may be due to frequent use of antimicrobials on chicken farms. This is supported by the observations of others. According to Lee et al. (2003), *Salmonella enterica* serovar Gallinarum isolates from chickens in South Korea were susceptible to most conventional antimicrobial agents in 1995, whereas, in 2001, many isolates were resistant or intermediately resistant to aminoglycosides. Antimicrobials, including aminoglycosides, are commonly used on commercial chicken farms in South Korea to prevent or treat fowl typhoid (Kang et al., 2010).

In the current study, all 10 *Salmonella* Gallinarum isolates from the organic farm and 13 of 16 (81.0%) from conventional farms showed intermediate resistance to streptomycin and it has become widespread in layer hens. Thus, the high

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**Table 1. Prevalence of *Salmonella* isolated from egg pools (20 eggs per pool) produced in either conventional or organic farms**

<table>
<thead>
<tr>
<th>Egg source</th>
<th>Farm type</th>
<th>Number of <em>Salmonella</em>-isolated egg pools/total number of pools tested (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm A</td>
<td>Conventional</td>
<td>3/50 (6.0)a</td>
</tr>
<tr>
<td>Farm B</td>
<td>Conventional</td>
<td>12/200 (6.0)a</td>
</tr>
<tr>
<td>Farm C</td>
<td>Conventional total</td>
<td>1/50 (2.0)a</td>
</tr>
<tr>
<td>Farm D</td>
<td>Organic</td>
<td>10/50 (20.0)b</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>26/350 (7.4)</td>
</tr>
</tbody>
</table>

a,b Different superscripts within a column indicate a statistically significant difference ($P < 0.05$).
frequency of antibiotic resistance in isolates from the organic farm may be a consequence of continued use of antimicrobials in chickens despite the introduction of the live vaccine in South Korea in 2001.

**Molecular Characterization by rep-PCR Fingerprinting**

In this study, we differentiated *Salmonella* isolates using the rep-PCR system (DiversiLab). We also used this analysis to determine the relationships among the *Salmonella* Gallinarum isolates, *Salmonella Enteritidis pig-1 strain*, and the vaccine strain, SG 9R (Figures 1 and 2). The rep-PCR pattern of *Salmonella Enteritidis* pig-1 strain was easily differentiated from *Salmonella Gallinarum* isolates with less than 75% similarity. The rep-PCR analysis identified 2 similar genetic groups among the *Salmonella* Gallinarum isolates, and all seemed to be either genetically identical or closely related, having a greater than 96% similarity. Interestingly, the vaccine strain (SG 9R) showed a notably different pattern, indicating that it is distinct from the field strains of *Salmonella Gallinarum*. The rep-PCR allowed discrimination between *Salmonella* isolates and the vaccine strain SG 9R, where there was a less than 86% similarity index. The dendrogram generated from the rep-PCR patterns and computer-generated virtual gel images are summarized in Figure 1.

All 26 of the salmonellae we isolated from egg contents in South Korea were *Salmonella* Gallinarum; there was no *Salmonella Enteritidis* (Figure 2). These data may indicate that there is an inverse relationship between the incidence of *Salmonella* Gallinarum infection in chickens and egg-associated *Salmonella Enteritidis* infections in humans. These results may be consistent with the hypothesis that *Salmonella Enteritidis* filled the ecologic niche vacated by the eradication of *Salmonella Gallinarum* from poultry, leading to an epidemic increase in human infections (Bäumler et al., 2000). This hypothesis has been supported by experimental evidence indicating that immunization with one *Salmonella* serovar can generate cross-immunity against a second serovar if both organisms have the same immunodominant O-antigen on their cell surface, such as the shared immunodominant O9-antigen of *Salmonella Gallinarum* and *Salmonella Enteritidis* (Rabsch et al., 2000).

In the present study, antibiotic susceptibility testing and molecular subtyping using rep-PCR confirmed that all 26 isolates are not the vaccine strain, SG 9R, which has been widely used in egg-producing chickens in South Korea since 2001. Although our results have demonstrated that rep-PCR can be used to reliably and rapidly differentiate field strains from the vaccine strain SG 9R, it has limited ability to discriminate between *Salmonella Gallinarum* wild-type isolates. It seems that *Salmonella* isolates of the same serovar and antibiotic resistance pattern are not readily distinguishable by rep-PCR. These results are in agreement with those of Wise et al. (2009). Therefore, a combination of additional genotyping methods, such as phage typing, seems to be needed to effectively distinguish *Salmonella* isolates of the same serovar.

Even though *Salmonella* Gallinarum has adapted to its avian host and rarely induces food poisoning in humans, it causes a significant poultry disease, fowl typhoid, which is responsible for considerable economic losses to the poultry industry (Hyeon et al., 2013). Even though fowl typhoid has been almost eradicated in Australia, North America, and most European coun-

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**Table 2. Antimicrobial resistance of *Salmonella* isolated from the contents of shell eggs**

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Organic (n = 10)</th>
<th>Conventional (n = 16)</th>
<th>Total (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>I</td>
<td>R</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ciprofloxacillin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>0 (0)</td>
<td>10 (100)a</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Sulfadiazine</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Amoxiclavul</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ciprofloxacillin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cefotaxim</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Enrofloxacillin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Norfloxacillin</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>10 (100)a</td>
<td>0 (0)</td>
<td>16 (100)b</td>
</tr>
</tbody>
</table>

a,b Different superscripts within a row indicate a significant difference (P < 0.05). (No. of antibiotic resistance/total no. of strain) × 100.

R, resistant; I, intermediately resistant; sulfa-trime, sulfamethoxazole-trimethoprim; amoxi-clavul, amoxicillin-clavulanic acid.
Figure 1. Dendrogram analysis and virtual gel image of DiversiLab automated repetitive sequence-based PCR fingerprinting analysis of the vaccine strain (SG 9R) and Salmonella isolates. Key to sample identification (ID): classified by farm type (O_#: isolates from organic eggs; N_#: isolates from nonorganic, conventional eggs); key to antimicrobial drugs: E, erythromycin-resistant isolates; S, streptomycin-resistant isolates; SI, streptomycin-intermediate-resistant isolates.
tries, eradication in other areas such as Africa, Asia, the Middle East, and Central and South America has proved extremely difficult (Silva et al., 1981b; Barrow, 1990; Kim et al., 1991; Wigley et al., 2005; Basnet et al., 2008). Several strategies aimed at reducing chicken infection by this bacterium have been employed, including establishment of hygiene standards, husbandry improvements, and the use of antibiotics and vaccines (Zhang-Barber et al., 1999; Beal et al., 2004). Various killed or live attenuated *Salmonella* strains have been tested in fowl model systems as potential vaccines (Barrow et al., 1990; Feberwee et al., 2000; Woodward et al., 2002). Although the use of live vaccine was authorized for commercial egg farms in South Korea, residual viru-

![Figure 2. Scatterplot arrangement of vaccine strain (SG 9R) and *Salmonella* strains isolated from egg pools produced in conventional or organic farms. The vaccine strain (SG 9R) and 26 *Salmonella* Gallinarum isolates (2 distinct genetic groups: R1 and R2) are arranged on the plot to show the relationship of each isolate to all other isolates. The gridlines give an approximate percentage of similarity.](https://academic.oup.com/ps/article-abstract/92/10/2789/1551224)
ence has been observed, and the protection provided has been insufficient (Bouzoubaa et al., 1989). *Salmo-
ella* Gallinarum is thought to invade the mononuclear phagocyte system within the macrophages of the liver and spleen (Barrow et al., 1994). That could be the main reason why *Salmonella* Gallinarum has persisted in commercial layer flocks in Korea even though the attenuated *Salmonella* Gallinarum strain 9R has been used as a commercial vaccine to control fowl typhoid (Lee et al., 2007). The persistence of *Salmonella* Gal-
inarum in commercial layer flocks also demonstrates the difficulty of controlling and the importance of pre-
venting infection. It implies that a national risk-based approach is absolutely needed to monitor and prevent infection throughout layer breeding and egg production. Further studies are also needed to assess the ef-
cacy of the current SG 9R vaccine.

In conclusion, 26 *Salmonella* Gallinarum were iso-
lated from 7,000 egg contents, with a higher prevalence rate in eggs from organic farm than in those from con-
ventional farms. However, no eggs tested positive for *Salmonella* Enteritidis, the most frequent *Salmo-
nera* serovar found in eggs in other countries including the United States (Patrick et al., 2004; Gast et al., 2009; Betancor et al., 2010).

**ACKNOWLEDGMENTS**

This research was supported by Mid-career Researcher Program (2012R1A2A2A-01015344) through the Na-
tional Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology, and by the Bio-industry Technology Development Pro-
gram of IPET (no. 112137-3) funded by the Ministry for Food, Agriculture, Forestry, and Fisheries. Jung-
Whan Chon and Kwang-Young Song were also partially supported by the Brain Korean 21 (BK21) Project from the Ministry of Education and Human Resources Development.

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