Emergence and Characterization of Foodborne Methicillin-Resistant *Staphylococcus aureus* in Korea

CHAE HONG RHEE and GUN-JO WOO*

Laboratory of Food Safety & Evaluation, Department of Food Bioscience & Technology, Korea University, Anam-dong, Seongbuk-gu, Seoul, 136-713, Korea

ABSTRACT

A total of 165 *Staphylococcus aureus* strains, isolated from different food samples between 2003 and 2006, were tested for antimicrobial susceptibility. The mecA-positive methicillin-resistant *S. aureus* (MRSA) strains were further characterized by testing for various virulence genes and by molecular typing with multilocus sequence typing and pulsed-field gel electrophoresis. Of the 165 *S. aureus* isolates, 150 strains (90.9%) were resistant to at least one antibiotic while no strain was resistant to vancomycin. Four strains were resistant to both oxacillin and cefoxitin and were mecA positive. The mecA-positive MRSA strains were isolated from raw meat and fish samples (two beef samples and two fish samples) and were resistant to β-lactam antibiotics. Based on multilocus sequence typing analysis, the isolates were assigned to sequence type 1 (ST1), ST72, and an undetermined ST (ST72 slv). All four MRSA isolates were shown to be enterotoxigenic. The ST1 MRSA isolate harbored the *sea-seg* gene combination and the ST72 and ST72 slv MRSA strains harbored the *sea-seh* and the *sea-seg-sei* gene combinations, respectively. However, none of the MRSA isolates had the genes for Panton-Valentine leukocidin, toxic shock syndrome toxin 1, and exfoliative toxins. The pulsed-field gel electrophoresis patterns of the ST72 isolates in our study were highly similar, even though they were isolated from food samples in different years and from different regions of Korea.

*Staphylococcus aureus* is considered to be one of the most common pathogens responsible for outbreaks of food poisoning. Methicillin-resistant *S. aureus* (MRSA) has become a significant and increasing cause of nosocomial infections and one of the most prevalent pathogens worldwide, with a significant economic impact on health care systems (22). Community-associated MRSA (CA-MRSA) isolates are an emerging concern. The evolution of CA-MRSA in persons with health care–associated risk factors was initially reported in the early 1980s among hospital employees in the United States (25).

Recently, MRSA has become a zoonotic issue since a particular MRSA strain of sequence type 398 (ST398) has been frequently detected from pigs and pig handlers. Several reports have demonstrated the elevated incidence of the prevalence of MRSA among pig handlers, which suggests a presumable regional emergence of CA-MRSA (13, 16, 23, 29). Moreover, there have been reports of MRSA in aquatic animals and of antimicrobial-resistant *S. aureus* in cases of fish handlers’ disease (1, 2). The increasing prevalence of antimicrobial-resistant *S. aureus*, including MRSA in farming animals and handlers, may play an important role in food safety and become a threat in the health care system.

The purpose of the present study was to determine the antimicrobial susceptibility of *S. aureus* isolates from various food samples and to characterize the foodborne MRSA isolates by evaluating their ability to produce virulence genes by multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE).

MATERIALS AND METHODS

*S. aureus* isolates. Between the years 2003 and 2006, 165 *S. aureus* strains were collected nationwide from various food samples by the National Antimicrobial Resistance Management Program in the Korea Food and Drug Administration. The isolates were further confirmed as *S. aureus* by VITEK 2 Compact (BioMérieux, Inc., Hazelwood, MO).

Antimicrobial susceptibility testing. The antimicrobial susceptibility profiles of the isolates were determined by the disk diffusion method on Mueller-Hinton agar (BD Diagnostic Systems, Sparks, MD) as standardized by the Clinical and Laboratory Standards Institute guidelines (6). The following antibiotic disks (BD Sensi-disc, BD, Sparks, MD) were tested: penicillin (10 U), oxacillin (1 μg), ampicillin (10 μg), gentamicin (10 μg), ciprofloxacin (5 μg), erythromycin (15 μg), clindamycin (2 μg), chloramphenicol (30 μg), vancomycin (30 μg), tetracycline (30 μg), and rifampin (5 μg). The MICs for all the strains were also examined by the agar dilution method on Mueller-Hinton agar to confirm antimicrobial susceptibilities, following Clinical and Laboratory Standards Institute guidelines (6). The MICs ranged from 0.06 to 256 μg/ml. The resistance rate was calculated as the ratio of the number of intermediate and resistant strains to the total number of strains. *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 were used as control strains to ensure the validity of the
susceptibility testing. Results were recorded after 18 to 24 h of incubation at 37°C and interpreted according to the breakpoints of each antibiotic as described in the Clinical and Laboratory Standards Institute instructions (6).

Detection of MRSA strains. Oxacillin-resistant S. aureus strains were further tested for cefoxitin (30 μg; BD Sensi-disc) resistance by disk diffusion (5), and the strains were also subjected to a PCR assay to detect the mecA gene by using a primer pair (forward, 5’-TGGCTATCGTGCACAATCG-3’; reverse, 5’-CTGGAACTGTGAGCAAGAG-3’) previously reported by Van nu ffel et al. (28). Chromosomal DNAs were extracted from the strains by the simple lysis method as previously described (4), and the PCR amplification was carried out on an iCycler thermal cycler (Bio-Rad, Hercules, CA) with initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 s, primer annealing at 60°C for 30 s, and extension at 72°C for 30 s. A final extension was performed at 72°C for 5 min. The PCR products were resolved by electrophoresis of 5 μl of the products on a 1.5% agarose gel (Promega, Madison, WI) at 100 V for 20 min and visualized by a gel documentation system (Bio-Rad). PFGE results were analyzed by InfoQuest FP Software (Bio-Rad). The resulting banding patterns were compared by using Dice coefficients with 1.0% band position tolerance.

RESULTS

Antimicrobial resistance of S. aureus isolates. A total of 165 S. aureus isolates were grouped into three categories based on food sample origins, which were livestock products (beef, pork, and chicken; n = 124), fishery products (n = 12), and retail processed foods (frozen meat products, and retail ready-to-eat food; n = 29). The food samples were obtained from eight different regions (Seoul, Gyeonggi Province, Incheon, Busan, Gwangju, Daejeon, Cheongju, and Jeju island in Korea). The 165 S. aureus isolates showed different antimicrobial resistance profiles. The great majority of them, 150 isolates (90.9%), were resistant to at least one antibiotic, and 33 isolates (20.0%) were resistant to at least three classes of antibiotic (data not shown). Overall, the isolates were observed to be mostly resistant to β-lactams such as penicillin (72.7%) and ampicillin (72.7%), while no strain was resistant to vancomycin or rifampin. A small percentage of the isolates demonstrated resistance to oxacillin and chloramphenicol (Table 1).

Identification of MRSA. Of the 165 S. aureus strains, 4 were concurrently resistant to oxacillin and cefoxitin (30 μg; BD Sensi-disc), and these strains were confirmed to harbor the mecA gene. Each mecA-positive strain was derived from different samples. Two of the strains were isolated from rockfish (KUSAU05070) and sea bass (KUSAU05081) in 2005, and two were isolated from beef (KUSAU06143 and KUSAU06181) in 2006.

Characteristics of MRSA isolates. Each mecA-positive strain was characterized, and the results were summarized in Table 2. In accordance with the Clinical and Laboratory Standards Institute guidelines (6), the four mecA-positive strains were concluded to be MRSA (MIC of oxacillin, ≥16 μg/ml). All MRSA strains were resistant to all β-lactams tested. For the two isolates from fish samples, the MICs of gentamicin were 64 to 128 μg/ml, but only one of the fish sample isolates was resistant to tetracycline. No resistance to erythromycin, clindamycin, vancomycin, chloramphenicol, and rifampin was observed.

The results of MLST analysis of the four mecA-positive strains were compared to a database of S. aureus STs.
### TABLE 1. Antimicrobial resistance of foodborne S. aureus isolates

<table>
<thead>
<tr>
<th>Samples (no. of isolates)</th>
<th>P</th>
<th>OX</th>
<th>AM</th>
<th>GM</th>
<th>CIP</th>
<th>E</th>
<th>CC</th>
<th>VA</th>
<th>C</th>
<th>TE</th>
<th>RA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Livestock products (124)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef (39)</td>
<td>33 (84.6)</td>
<td>2 (5.1)</td>
<td>33 (84.6)</td>
<td>2 (5.1)</td>
<td>6 (15.4)</td>
<td>10 (25.6)</td>
<td>9 (23.1)</td>
<td>0 (0.0)</td>
<td>1 (2.6)</td>
<td>10 (25.6)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Pork (36)</td>
<td>31 (86.1)</td>
<td>0 (0.0)</td>
<td>31 (86.1)</td>
<td>5 (13.9)</td>
<td>4 (11.1)</td>
<td>11 (30.6)</td>
<td>14 (38.9)</td>
<td>0 (0.0)</td>
<td>3 (8.3)</td>
<td>23 (63.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Chicken (49)</td>
<td>24 (49.0)</td>
<td>0 (0.0)</td>
<td>25 (51.0)</td>
<td>22 (44.9)</td>
<td>14 (28.6)</td>
<td>6 (12.2)</td>
<td>1 (2.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>26 (53.1)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Subtotal avg</strong></td>
<td>88 (71.0)</td>
<td>2 (1.6)</td>
<td>89 (71.8)</td>
<td>29 (23.4)</td>
<td>24 (19.4)</td>
<td>27 (21.8)</td>
<td>24 (19.4)</td>
<td>0 (0.0)</td>
<td>4 (3.2)</td>
<td>59 (47.6)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Marine products (12)</strong></td>
<td>10 (83.3)</td>
<td>2 (16.7)</td>
<td>10 (83.3)</td>
<td>3 (25.0)</td>
<td>0 (0.0)</td>
<td>1 (8.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (8.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Processed foods (29)</strong></td>
<td>22 (75.9)</td>
<td>0 (0.0)</td>
<td>21 (72.4)</td>
<td>1 (3.4)</td>
<td>1 (3.4)</td>
<td>6 (13.8)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (3.4)</td>
<td>2 (6.9)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Total avg</strong></td>
<td>120 (72.7)</td>
<td>4 (2.4)</td>
<td>120 (72.7)</td>
<td>33 (20.0)</td>
<td>25 (15.2)</td>
<td>34 (20.6)</td>
<td>24 (14.5)</td>
<td>0 (0.0)</td>
<td>5 (3.0)</td>
<td>62 (37.6)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

*a* P, penicillin; OX, oxacillin; AM, ampicillin; GM, gentamicin; CIP, ciprofloxacin; E, erythromycin; CC, clindamycin; VA, vancomycin; C, chloramphenicol; TE, tetracycline; RA, rifampin.

### TABLE 2. Microbiological characteristics of foodborne MRSA isolates in Korea

<table>
<thead>
<tr>
<th>Isolate Source</th>
<th>Toxin genotype</th>
<th>MLST</th>
<th>MIC (µg/ml) (antimicrobial susceptibility)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SEs</td>
<td>TSST-1</td>
<td>ETs</td>
</tr>
<tr>
<td>KUSAU05070 Fish (rockfish)</td>
<td>seg, sei</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>KUSAU05081 Fish (sea bass)</td>
<td>sea, seh</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>KUSAU06143 Beef</td>
<td>seg, sei</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>KUSAU06181 Beef</td>
<td>sea, seg, sei</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup> SEs, enterotoxins; TSST-1, toxic shock syndrome toxin 1; ETs, exfoliative toxins; PVL, Panton-Valentine leukocidin; P, penicillin; OX, oxacillin; AM, ampicillin; GM, gentamicin; CIP, ciprofloxacin; E, erythromycin; CC, clindamycin; VA, vancomycin; C, chloramphenicol; TE, tetracycline; RA, rifampin.
<sup>b</sup> Allelic profiles are given in the following order: arcC-aroE-glpF-gmk-pta-tpi-yqiL.
<sup>c</sup> —, not detected.
available on-line (http://saureus.mlst.net). One strain was identified as ST1, and two strains were identified as ST72. The remaining strain was determined to be a single-locus variant of ST72 (ST72 slv), differing from this genotype by a single point mutation in the gene encoding guanylate kinase (gmk). For the ST1 MRSA strain (KUSAU05081) all β-lactams had higher MICs than for the other strains. The two MRSA isolates from fish samples were resistant to gentamicin, and of the two, one was also resistant to tetracycline.

All MRSA isolates possessed more than two types of SE-encoding genes, while none of them carried the genes encoding PVL, TSST-1, or ETs. The SE genes detected were sea-seg-sei, seg-sei, and sea-seh.

The two ST72 isolates displayed 85.7% similarity despite being isolated from different food samples in different years, and the ST72 and ST72 slv isolates showed a similarity of 78.6% by PFGE (Fig. 1).

**DISCUSSION**

Antimicrobial resistance in many bacterial pathogens responsible for community-acquired and nosocomial infections is becoming a major concern. Antibiotic resistance can be spread by antibiotic residues in food products, through the transfer of resistant foodborne pathogens, or through the ingestion of resistant strains of the original food microflora and resistance transfer to pathogenic microorganisms (22). Many researchers have reported resistant strains of *S. aureus* isolated from various food samples in different countries (17–19, 21, 22, 24, 27). In our study, the rate of resistant strains was high (90.9%), and resistance to members of the penicillin family, penicillin (72.7%) and ampicillin (72.7%), was the most frequently observed. This is in good agreement with the results of previous studies in Portugal and Italy (21, 22). High sales of penicillins and tetracyclines to be administered to food-producing animals in Korea (Korea Food and Drug Administration, 2009, National Antimicrobial Resistance Management Program annual report (14)) seemed to be associated with the high levels of resistance to penicillin. Fifteen (9.1%) *S. aureus* isolates were sensitive to all the tested antibiotics, and 33 (20.0%) were resistant to three or more classes of antibiotics. These results show that multidrug resistance among foodborne *S. aureus* is already significant.

Among the 165 *S. aureus* isolates studied here, 4 strains were resistant to oxacillin and cefoxitin and were positive for the meca gene, a fairly low percentage (2.4%). The rate of MRSA among *S. aureus* isolates in our study is comparable to rates reported by other studies. MRSA has been isolated from various food samples in other countries. However, the percentage of MRSA strains detected from foods of animal origin varies widely, from 3.75% (17) in Italy to 18.1% in the United States (24). A recent Dutch study showed a high percentage of MRSA isolated from raw meat, with the highest prevalence in poultry meat (7).

Our detection of enterotoxin genes in the four MRSA strains agrees with a 2007 report showing the coexistence of the toxin genes *seg* and *sei* in foodborne *S. aureus* in Korea (18). However, TSST-1–, ET–, and PVL-producing genes that cause toxin-mediated diseases were not detected in this study (Table 2). Cha et al. (5) reported that the *sea* gene was detected in the majority of isolates (91.9%) associated with staphylococcal food poisoning, and an ST1 strain with the *sea-seh* gene combination was the most epidemic clone found in staphylococcal food poisoning incidents in Korea. The ST1 MRSA strain in this study also carries the *sea-seh* gene combination, and the result matches that reported in the study by Cha et al. (5). The results suggest the evidence of considerable transmission of antimicrobial-resistant pathogens and the origin of staphylococcal food poisoning in Korea. ST1 MRSA was reported as the most common clone identified in the homeless people and injection drug users who shared the same homeless shelter, which suggests a possible focus of community transmission (19). However, further characterization study is required to confirm the relatedness with our clone.

In Korea, many reports on CA-MRSA infections show that ST72 is the major genotype in CA-MRSA isolates (3, 11), which distinguishes them from CA-MRSA isolates in other countries. ST72 MRSA was the cause of a community-associated outbreak between 2004 and 2005 in Korea (14, 20) and of a clinically associated outbreak in Brazil (26).

The ST72 and ST72 slv MRSA strains from this study contained *seg-sei* gene and *sea-seg-sei* gene combinations, respectively, which were frequently detected genes in staphylococcal food poisoning (5). MRSA commonly carries enterotoxin genes, but there has been only one outbreak report of food intoxication due to MRSA (9). The two MRSA strains isolated from raw fish samples had high MICs for gentamicin (64 to 128 μg/ml), presumably caused by the frequent use of gentamicin in aquaculture in Korea.
The two ST72 MRSA isolates displayed a close genetic relationship by PFGE patterns, even though they were isolated from different food samples in different years and regions. The 2009 annual report of the National Antimicrobial Resistance Management Program in Korea (14) showed that the ST72 MRSA strains, a typical CA-MRSA in Korea, were also isolated from the nasal cavity of pigs. Taken together, our findings suggest a national distribution of ST1 and ST72 not only in the community or hospital setting but also in food products in Korea. Thus, further epidemiologic investigation of the transmission pathways for these MRSA strains is essential.

Other MRSA strains have also been observed. Suda-gidan and Aydin (27) identified three ST152 S. aureus isolates from different food samples. ST5, ST8, and ST398 MRSA were also isolated from raw food samples of food-producing animals (13, 24). However, because reports on the presence, possible origin, and molecular typing of foodborne MRSA in foods are limited, it is still unclear what role is played by foodborne MRSA and what the sources of contamination are (17).

To our knowledge, our study is the first to provide MLST types of foodborne MRSA in Korea. The results of our study highlight that, although the prevalence of MRSA in food is currently low, the risk of its transmission through the food chain cannot be disregarded (13), especially in uncooked meat and raw fish. Moreover, this work provides further support for the hypothesis that MRSA can be cross-contaminated between humans and foods and emphasizes the importance of improving hygiene in food production practices as a countermeasure to limit the spread of antimicrobial-resistant organisms via foods.

ACKNOWLEDGMENTS

This work was supported by a Korea University Grant (K0822761) and a grant from the National Antimicrobial Resistance Management Program (08072NARMPI150) of the Korea Food and Drug Administration. We also thank the Korea University Food Safety Hall and Institute of Food and Biomedicine Safety for allowing the use of their equipment and facilities.

REFERENCES


