Commercially Distributed Meat as a Potential Vehicle for Community-Acquired Methicillin-Resistant *Staphylococcus aureus*

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The incidence of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infection has been increasing; however, the sources of infection remain unclear. Therefore, we investigated the involvement of meat as a possible mediator of CA-MRSA infection. We examined the distribution of MRSA strains in commercially distributed raw meat samples (n = 197) and diarrheal stool samples of outpatients (n = 1,287) that were collected in Oita Prefecture, Japan, between 2003 and 2009 for routine legal inspections. Fourteen MRSA strains were isolated from three meat and 11 stool samples. Among these, seven isolates from three meat and four stool samples exhibited the same epidemiological marker profiles [coagulase type III, staphylococcal enterotoxin C, staphylococcal chromosomal cassette mec (SCCmec) type IV, ST8, spa type 606 (11767), and toxic shock syndrome toxin-1 (TSST-1) producing type]. Furthermore, of the seven strains, three isolates from two meat samples and one stool sample collected in 2007 exhibited completely identical characteristics with respect to phage open reading frame (ORF) typing, pulsed-field gel electrophoresis, and drug susceptibility profiles. The results suggest that commercially distributed meat could play a role in the prevalence of CA-MRSA in the community.

Methicillin-resistant *Staphylococcus aureus* (MRSA) infections have been significant causes of nosocomial infections. The U.S. Centers for Disease Control and Prevention reported the first non-nosocomial case of MRSA in 1981. Thereafter, such MRSA strains have been called community-acquired MRSA (CA-MRSA) (2). Since the first case was reported, many reports have discussed the isolation of MRSA from people infected outside hospitals. CA-MRSA has been distinguished from hospital-acquired MRSA (HA-MRSA) on the basis of contact with health care settings. However, CA-MRSA has recently been detected in individuals from health care settings and in otherwise healthy infants and adolescent students (3).

Initially, it was believed that this situation was because of the dispersion of HA-MRSA from health care settings through patients and health care workers. However, based on the results of statistical and molecular genetics analyses, Naimi et al. reported that the source of most MRSA strains prevalent in the community differs from that of MRSA strains causing nosocomial infections (21). Thus, the source of CA-MRSA remains unclear. In Europe and North America, the common characteristics of CA-MRSA strains are similar to those described by Naimi et al.; namely, CA-MRSA strains carry the Panton-Valentine leukocidin (PVL) gene and staphylococcal chromosomal cassette mec (SCCmec) type IV (35) and are sensitive to antibiotics other than penicillin/cephalosporin derivatives (37). On the other hand, recent reports from Asian countries, including Japan, indicate that PVL-positive strains are far less prevalent (12, 34, 37).

It has recently been reported that domestic pets, livestock, wild birds, and other animals carry MRSA (3, 7). Furthermore, MRSA has been isolated from retail meat products, milk and meat samples, and meat industry workers. Antimicrobial agents are widely used as feed additives in livestock farms, and their use is known to result in favorable breeding grounds for MRSA (33, 36). There have been reports from Japan, South Korea, Spain, The Netherlands, and the United States (4, 7, 13, 15–17, 28) regarding MRSA contamination of meat, focusing on raw meat in retail markets. This fact strongly suggests that consumption of MRSA-contaminated foods would be responsible for the prevalence of CA-MRSA in the community. However, there are almost no detailed reports from the viewpoint of CA-MRSA contamination of foods. Moreover, if people eat undercooked MRSA-contaminated meat, they may excrete MRSA in their stools, and MRSA may spread widely in the community through their hands. CA-MRSA strains have most frequently been isolated from human skin/soft tissue (5); however, the distribution of CA-MRSA in diarrheal stools of outpatients has not been examined. Furthermore, the relationship between meat- and stool-derived MRSA strains has not been studied. For public health, our institute routinely inspects contamination states of meats with *S. aureus* according to a food hygiene survey plan of Oita Prefecture. Diarrheal stool samples of outpatients are also tested to determine the causative agents including *S. aureus*. To know the distribution of CA-MRSA in Oita Prefecture and to elucidate the transmission route, we performed detailed characterization of these *S. aureus* isolates.

**MATERIALS AND METHODS**

**Samples.** In total, 1,484 samples (197 meats and 1,287 diarrheal stools of outpatients) were collected in Oita Prefecture, Japan, from April 2003 to March 2009 for legal inspections (Table 1). The meat samples were raw intact muscles obtained from commercially distributed meat in retail markets according to the food hygiene survey plan of Oita Prefecture. Approximately 30 meat samples were brought to our institute for annual monitoring. These samples were inspected for *S. aureus* contamination. The main meat samples comprised chicken, beef, and pork meats, which are widely distributed and abundantly consumed in our prefecture, whereas venison and wild boar meats are game meats. On the other hand, human diarrheal stool samples were also brought to our institute for inspection when...
symptoms occurred outside the hospitals. To exclude HA-MRSA, the stool samples were obtained within 48 h after a medical doctor was consulted. Examination was started on the day on which the samples were brought to our institute. Until the examination, all the samples were stored at 4°C.

**Isolation and characterization of *S. aureus*, MRSA, and diarrhea-causing bacteria.** Diarrhea-causing bacteria were isolated according to conventional methods (see Fig. S1 and S2 in the supplemental material). *S. aureus* was identified by mannitol fermentation and egg yolk reactions on mannitol salt egg yolk (MSEY) agar medium (Sysmex bioMérieux Co. Ltd., Tokyo, Japan), followed by Gram staining, catalase and coagulase tests, and PCR for femB (27). MRSA was identified by growth on oxacillin resistance screening agar (Oxoid, England) and by the presence of mecA using PCR.

**Coagulase and SE typing and TSST-1 production.** Coagulase typing was performed using staphylocoagulase antiserum (Denka Seiken Co. Ltd., Tokyo, Japan). Staphylococcal enterotoxin (SE) typing was performed using staphylocoagulase antiserum (Denka Seiken Co. Ltd., Tokyo, Japan), followed by Gram staining, catalase and coagulase tests, and PCR for femB (27). MRSA was identified by growth on oxacillin resistance screening agar (Oxoid, England) and by the presence of mecA using PCR.

**DNA extraction and PCR.** The strains incubated overnight on nutrient agar medium were suspended in 500 μl of 3% (wt/vol) Chelex (Chelex 100 resin, 200 to 400 dry mesh size; Bio-Rad Laboratories, Inc., Hercules, CA) in TE buffer (10 mM Tris-HCl [pH 8.0] and 1 mM EDTA) and incubated at 56°C for 30 min with occasional shaking. The samples were then boiled for 8 min and centrifuged for 5 min at 12,000 rpm. The supernatants were used as DNA templates for the following PCR assays.

**mecA and femB were detected by multiple PCR based on the method used by Higashine et al. (10) (see Table S1 in the supplemental material).** tst and the SE genes (sea-see and seg) were examined using primer sets produced by Takara Bio, Inc. (Otsu, Japan). The genes downstream of seg, other than sea-sec, were verified according to the method described by Omoe et al. (24). The presence of the PVL gene was confirmed by PCR performed according to the method described by Lina et al. (18). The exfoliative toxin (ET) genes (eta and etb) were confirmed by multiplex PCR based on the method used by Fujimoto and Kojima (8) (see Table S1).

**SCC mec and staphylococcal protein A (spa) gene typing were performed according to previous reports (23, 31).** The spa type was determined using the eGenomics web server (eGenomics, Inc., New York, NY) and Ridom web server (http://spaserver2.ridom.de/) (9, 14, 34). Multilocus sequence typing (MLST) was performed as described previously (6, 9, 14, 34). Phage open reading frame (ORF) typing (POT) was performed using a Cica Geneus Staph POT kit for *S. aureus* (Kanto Chemical Co., Inc., Tokyo, Japan) (32).

**PFGE.** Pulsed-field gel electrophoresis (PFGE) was performed by modifying the method described by Ichiyama et al. (11). The restriction enzyme SmaI (30 units/sample) (Takara Bio Inc.) was used, and the electrophoresis conditions were a pulse time of 5.3 to 34.9 s, buffer temperature of 12°C, and migration time of 20 h.

**Drug susceptibility test.** Drug susceptibility was assayed using a dry plate (Eiken Chemical Co., Ltd.). The following 15 antibiotics were tested: oxacillin (OXA), ampicillin (AMP), cefazolin (CZF), cefmetazole (CMZ), imipenem (IPM), gentamicin (GEN), minocycline (MIN), cefoxitin (FOX), erythromycin (ERY), clindamycin (CLI), vancomycin (VAN), teicoplanin (TEC), linezolid (LZD), trimethoprim-sulfamethoxazole (SXT), and levofloxacin (LVX).

**Epidemiological background.** Workers who possibly handled MRSA-positive meat were interviewed regarding hospital visits and doctor consultations. Patients with MRSA-positive diarrhea were interviewed regarding hospitalization.

### RESULTS

**Isolation of *S. aureus* and MRSA.** In total, 197 meat samples (nine different types of meat) were examined (Table 1). Among these, 56 *S. aureus* strains were recovered from 47 meat samples (23.9%, 47/197; six types of meat) (Table 1). Three MRSA strains were isolated from two chicken samples and one duck meat sample. The rate of MRSA contamination of meats was 1.5% (3/197), and MRSA comprised 5.4% of the *S. aureus* isolates (3/56). Among the 1,287 stool samples tested, 189 *S. aureus* strains were isolated from 174 samples (13.5%, 174/1,287). Among these, 11 were MRSA strains. The prevalence rate of MRSA among the human diarrheal stool samples was 0.9% (11/1,287), and MRSA comprised 5.8% of the *S. aureus* isolates (11/189).

**Coagulase and SE typing of *S. aureus* and MRSA.** Both the meat- and stool-derived *S. aureus* strains exhibited a wide distribution of coagulase types, excluding the absence of coagulase type I among the meat-derived strains (Tables 2 and 3). Coagulase types II, III, IV, V, VII, and UT were detected in more than 10% of both the meat- and stool-derived isolates, whereas coagulase types I, VI, and VIII were detected in less than 10%. All three MRSA isolates from the meat samples expressed coagulase type III, and 11 MRSA isolates from the stool samples expressed coagulase types I (five isolates), II (two isolates), and III (four isolates).

Concerning the SE type, the SE-nonproducing type was most frequently detected in both the meat- and stool-derived isolates (71.4% and 63.5%, respectively). All three MRSA isolates from the meat samples exhibited SEC, whereas the 11 MRSA isolates from the stool samples exhibited SEC (five isolates) or SEB and SEC (one isolate) or were nontoxigenic isolates (SE+; five isolates).

**Characterization of the 14 MRSA strains.** None of the 14 MRSA strains carried ET genes (eta and etb) or the PVL gene (see Table S2 in the supplemental material). Seven coagulase type III isolates (meat-derived isolates F1 to F3 and stool-derived isolates S1 to S4) showed identical results except with regard to the SE gene. They produced SEC and TSST-1 and carried the corresponding genes (sec, sel, and tst). Moreover, all these strains were sequence type 8 (ST8)/SCCmecIV/Spa type 606 (11767). On the other hand, the coagulase type I isolates (S5 to S9) showed the same results except with regard to the spa type. They did not produce SE and TSST-1 but carried sel. Furthermore, the isolates, except isolate S6, showed ST89/SCCmecI/spa type 416 (1375).

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**TABLE 1 Distribution of *S. aureus* in meats and human diarrheas**

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Total no. of samples</th>
<th>No. of <em>S. aureus</em>-positive samples (%)</th>
<th>No. of <em>S. aureus</em> isolates</th>
<th>No. of MRSA-positive samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td>73</td>
<td>25</td>
<td>28</td>
<td>2</td>
</tr>
<tr>
<td>Beef</td>
<td>55</td>
<td>7</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Pork</td>
<td>34</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Venison</td>
<td>21</td>
<td>9</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Wild boar</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Horse flesh</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Game fowl</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Duck</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Pheasant</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total for group</td>
<td>197</td>
<td>47 (23.9)</td>
<td>56</td>
<td>3 (1.5)</td>
</tr>
<tr>
<td>Human diarrheal stool</td>
<td>1,287</td>
<td>174 (13.5)</td>
<td>189</td>
<td>11 (0.9)</td>
</tr>
<tr>
<td>Overall total</td>
<td>1,484</td>
<td>221 (14.9)</td>
<td>245</td>
<td>14 (0.9)</td>
</tr>
</tbody>
</table>
The POT pattern of the seven coagulase type III/SEC isolates (F1 to F3 and S1 to S4) was 106 (Table 4). Among these, isolates F1, F3, and S3 exhibited completely identical POT patterns (106-9-80). The POT pattern of five coagulase type I/SEC/H11002 isolates (S5 to S9) was 104. Among these, three isolates (S5, S6, and S9) were 104-16-0 and two (S7 and S8) were 104-24-80.

The PFGE patterns were roughly classified into four types (A to D) (Fig. 1 and Table 4). The seven coagulase type III/SEC isolates (F1 to F3 and S1 to S4) showed the A type (A1 to A4), whereas the five coagulase type I/SEC/H11002 isolates (S5 to S9) showed the B type (B1 to B5). These four types differed from each other; however, the A or the B type showed analogous patterns within itself. Four of the seven isolates with the A type (F1, F3, S1, and S3) exhibited an identical A1 type.

Susceptibility to 15 antibiotics was examined. Twelve MRSA isolates (F1 to F3 and S1 to S9) were resistant to three to five antibiotics, including OXA, AMP, and GEN (Table 4). They were sensitive to antimicrobial agents other than beta-lactams and GEN. The seven coagulase type III/SEC isolates were resistant to four (four strains) or five antibiotics (three strains), and the five coagulase type I/SEC isolates showed resistance to three (two strains), four (two strains), or five antibiotics (one strain). The remaining two coagulase type II isolates (S10 and S11) were multidrug resistant (7 and 10 drugs, respectively). The three MRSA isolates (F1, F3, and S3) had identical drug susceptibility profiles.

Epidemiological background. The three meat samples from which the coagulase type III MRSA strains were isolated were collected in 2007 (Table 4); however, epidemiological investigation indicated nothing in common among the collection dates, breeding farms, meat processing plants, collection sites (vendors), and the meat processing and vendor workers. Furthermore, an interview of workers who handled the MRSA-positive meat confirmed that no worker visited a hospital around the time of sample collection. Among the 11 stool-derived strains, nine strains (S1 to S9) were isolated from young people (1 to 16 years old) without a history of hospitalization in the previous year. Conversely, the two strains (S10 and S11) were isolated from elderly patients (81 and 96 years old, respectively) living in a long-term care facility.

Five MRSA strains (F1, F2, S4, S5, and S7) were simultaneously isolated with *C. jejuni*. Strain S1 was isolated together with enterohemorrhagic *Escherichia coli* serotype O111: HNM (Shiga toxin 1-producing type), and strains S9, S10, and S11 were isolated with *Norovirus* genogroup I/8, *Salmonella* sp., and *Clostridium perfringens*, respectively.

**DISCUSSION**

There have been many reports regarding MRSA isolation from food (4, 7, 13, 20, 28); however, no report has compared human stool- and food-derived strains. Our study demonstrated the identical genetic fingerprint among strains derived from these different environments.
ent sources. This study showed that seven coagulase type III/SEC MRSA isolates from three meats and four diarrheal stools of 3- to 16-year-old outpatients had the same characteristics, namely, SCCmec type IV, ST8, spa type 606 (t1767), TSST-1 positive, sec and sel positive, PVL gene negative, and eta and etb negative. This study suggests that these seven strains had the same genotype. The epidemiological background indicated that the seven strains were unrelated to nosocomial infections. Indeed, they were highly sen-

TABLE 4 Molecular epidemiology of the MRSA isolates and epidemiological information of the MRSA-positive specimens

<table>
<thead>
<tr>
<th>Isolate type and no.</th>
<th>Origin</th>
<th>Collection date (mo/yr)</th>
<th>POT pattern</th>
<th>PFGE pattern</th>
<th>Drug resistance profile</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat-derived strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human diarrheal stool-derived strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>Male (4)</td>
<td>8/2003</td>
<td>106-45-112</td>
<td>A1</td>
<td>OXA/AMP/CFZ/FOX/GEN</td>
<td>EHEC O111</td>
</tr>
<tr>
<td>S3</td>
<td>Female (16)*</td>
<td>12/2007</td>
<td>106-9-80</td>
<td>A1</td>
<td>OXA/AMP/FOX/GEN</td>
<td></td>
</tr>
<tr>
<td>S4</td>
<td>Female (3)</td>
<td>4/2008</td>
<td>106-43-34</td>
<td>A4</td>
<td>OXA/AMP/FOX/GEN</td>
<td>C. jejuni</td>
</tr>
<tr>
<td>S5</td>
<td>Male (10)</td>
<td>12/2003</td>
<td>104-16-0</td>
<td>B5</td>
<td>OXA/AMP/FOX/GEN/ERY</td>
<td>C. jejuni</td>
</tr>
<tr>
<td>S6</td>
<td>Male (1)</td>
<td>9/2004</td>
<td>104-16-0</td>
<td>B1</td>
<td>OXA/AMP/GEN</td>
<td></td>
</tr>
<tr>
<td>S7</td>
<td>Male (14)</td>
<td>11/2004</td>
<td>104-24-80</td>
<td>B4</td>
<td>OXA/AMP/GEN</td>
<td>C. jejuni</td>
</tr>
<tr>
<td>S8</td>
<td>Female (4)</td>
<td>4/2005</td>
<td>104-24-80</td>
<td>B3</td>
<td>OXA/AMP/GEN/ERY</td>
<td></td>
</tr>
<tr>
<td>S9</td>
<td>Male (10)</td>
<td>11/2004</td>
<td>104-16-0</td>
<td>B2</td>
<td>OXA/AMP/FOX/GEN</td>
<td>Norovirus genogroup I/8</td>
</tr>
<tr>
<td>S10</td>
<td>Female (81)</td>
<td>9/2006</td>
<td>93-154-119</td>
<td>C</td>
<td>OXA/AMP/CFZ/CMZ/IPM/GEN/FOX/ERY/CLI/LVX</td>
<td>Salmonella</td>
</tr>
<tr>
<td>S11</td>
<td>Female (96)</td>
<td>2/2009</td>
<td>93-142-111</td>
<td>D</td>
<td>OXA/AMP/GEN/MIN/ERY/CLI/LVX</td>
<td>Clostridium perfringens (alpha toxin positive)</td>
</tr>
</tbody>
</table>

* F, meat derived, S, human stool derived.

**OXA, oxacillin; AMP, ampicillin; CFZ, cefazolin; CMZ, cefmetazole; IPM, imipenem; GEN, gentamicin; MIN, minocycline; FOX, cefoxitin; ERY, erythromycin; CLI, clindamycin; VAN, vancomycin; TEC, teicoplanin; LZD, linezolid; SXT, trimethoprim-sulfamethoxazole; LVX, levofloxacin.

For human-derived isolates, the age of the patient is given in parentheses.

High school student.

FIG 1 PFGE patterns of SmaI-digested genomic fragments of 14 MRSA isolates. Lane M, Salmonella enterica serovar Braenderup H9812 as a reference molecular size marker; lane 1, isolate F1 (pattern A1); lane 2, isolate F2 (pattern A2); lane 3, isolate F3 (pattern A1); lane 4, isolate S1 (pattern A1); lane 5, isolate S2 (pattern A3); lane 6, isolate S3 (pattern A1); lane 7, isolate S4 (pattern A4); lane 8, isolate S5 (pattern B5); lane 9, isolate S6 (pattern B1); lane 10, isolate S7 (pattern B4); lane 11, isolate S8 (pattern B3); lane 12, isolate S9 (pattern B2); lane 13, isolate S10 (pattern C); lane 14, isolate S11 (pattern D).
sitive to antibiotics. A characteristic of most CA-MRSA strains isolated in Japan is the absence of the PVL gene (30, 33, 34, 37). Considering these characteristics and the background, the seven strains can be regarded as CA-MRSA. Concerning the coagulase type and SE type of MRSA isolates from meat, Kitai et al. reported the first detection of two MRSA strains from commercially distributed meat (444 specimens derived from chicken) in Japan (13), both of which were coagulase type III and SEC and SE⁺. In addition, Murakami et al. (20) reported the detection of 42 MRSA isolates from 19 of 9,844 specimens collected from food ingredients, foods, and cooking facilities and reported that coagulase type III was the most abundant type among the MRSA isolates (76.2%, or 32/42). To confirm the identity, we used PFGE and POT, two different genotyping methods in principle, thereby increasing confidence in the high degree of identity among the MRSA strains. These methods revealed that the three coagulase type III/SEC MRSA isolates from the human stool (S3) and meat samples (F1 and F3), all of which were isolated in 2007, exhibited a high degree of identity. Whether the patient from whom strain S3 was isolated acquired MRSA through the consumption of contaminated meat is unclear. However, the patient probably did not participate in MRSA contamination of the meats (F1 and F3) because she was a high school student and not a worker handling meats. Additionally, strain S1, collected in 2003 from human stool, showed almost identical characteristics to strains F1 and F3. This suggested that a similar MRSA strain had already been distributed among the community in 2003.

In this study, we found that two MRSA isolates from chicken meats (F1 and F2) and three from diarrheal stools (S4, S5, and S7) were isolated together with C. jejuni. A high rate of contamination with C. jejuni with S. aureus in chicken meat and diarrheal stools (unpublished observations); however, whether the S. aureus isolates from diarrheal stools were MRSA was not determined. The reports and the current observation in this study suggest that some MRSA carriers or infected humans might acquire MRSA via the consumption of contaminated meat.

Whether CA-MRSA emerged in farms or other places such as meat processing factories remains to be determined. In any case, if these resistant bacteria are consumed by people and if antibiotic treatment is ineffective, it is highly possible that these bacteria will become established pathogens in humans. A much wider region could be affected by the commercial distribution of meats than by nosocomial infections. The consumption of contaminated meat should be included in the consideration of public health measures.

**REFERENCES**


