Isolation and Characterization of Methicillin-Resistant Staphylococcus aureus Strains from Louisiana Retail Meats

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We investigated the prevalence of Staphylococcus aureus and methicillin-resistant S. aureus (MRSA) in 120 retail meat samples from 30 grocery stores in Baton Rouge, LA. S. aureus strains were recovered from 45.6% of pork samples and 20% of beef samples, whereas MRSA strains were isolated from six meat samples (five pork samples and one beef sample). The MRSA isolates were of two strain types (clones), one harboring Panton-Valentine leucocidin and belonging to pulsed-field gel electrophoresis type USA300 and the other one belonging to USA100.

Community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) has emerged as a major public health concern worldwide (8). In the United States, among 8,987 cases of invasive MRSA reported in nine communities in 2005, 1,234 (13.7%) were due to CA-MRSA, and the remainder involved health care-associated (HA [85%]) and unclassified (1.3%) infections (10). Among the eight pulsed-field gel electrophoresis (PFGE) types (USA100 to USA800) originally identified in the United States, USA300 and USA400 have been associated with CA-MRSA infections and the remaining types have been associated with HA-MRSA (14). In particular, USA300 is the most common CA-MRSA clone in the United States (8, 15), and common HA-MRSA clones include USA100, USA800, and USA700 (16). The widely recognized association of CA-MRSA with type IV staphylococcal cassette chromosome mec (SCCmec) and the genes encoding Panton-Valentine leucocidin (PVL) has greatly facilitated strain differentiation between CA-MRSA and HA-MRSA (4).

Recent surveys conducted in The Netherlands and Canada have revealed a high prevalence (20 to 40%) of MRSA in pigs (2, 9). The pig-related MRSA strains possessed a distinct multilocus sequence type (ST398) and were nontypeable by SmaI PFGE (17, 18). Transmission of MRSA from pigs to pig farmers and their families has been documented in Europe (20). Thus, concerns have been raised that MRSA strains of animal origin could potentially enter the food chain and jeopardize the health of individuals handling meats (21). Researchers in The Netherlands have reported the isolation of a pig-related MRSA clone in Dutch meats (19). We undertook this study to determine the prevalence of S. aureus and MRSA in raw meat products which were collected in Baton Rouge, LA.

Isolation and characterization of S. aureus and MRSA. A total of 120 raw meat product samples (pork, n = 90; beef, n = 30) were randomly collected from 30 retail grocery stores of seven supermarket chains in Baton Rouge, LA. Sampling visits were made weekly for 6 weeks (February to March 2008). On each sampling day, five stores were chosen based on geographical proximity to each other. Three prepackaged pork chops and one beef steak in the refrigerated fresh meat section were collected from each store. After the exterior surface of the package was cleaned with paper towels moistened with 70% alcohol, the meats were mixed with equal volumes of buffered peptone water (BD Diagnostic Systems, Sparks, MD) and aseptically massaged for 5 min. A 50-ml aliquot of the rinse was enriched in an equal volume of double-strength enrichment broth (Trypticase soy broth supplemented with 10% NaCl and 1% sodium pyruvate). After 24 h of incubation at 35°C, the enrichment broth was streaked in duplicate on Baird-Parker (BP) medium and spread-plated on BP with cefoxitin (4 μg/ml [Sigma, St. Louis, MO]). Following 48 h of incubation, three to six presumptive S. aureus and MRSA colonies per meat sample (black colonies surrounded by 2- to 5-mm clear zones) were transferred to Trypticase soy agar plates followed by confirmation by a tube coagulase test (Remel, Lenexa, KS).

Confirmation of S. aureus and MRSA was conducted using a multiplex PCR for the species-specific 442-bp fragment with unknown coding potential (13) and mecA encoding a penicillin-binding protein with a low affinity for β-lactams (3). MRSA isolates were then characterized by antimicrobial susceptibility testing using broth microdilution in accordance with Clinical and Laboratory Standards Institute guidelines (1), PFGE with SmaI digestion (14), detection of the PVL gene by PCR (12), and characterization of the SCCmec type (22). Multilocus sequence typing (MLST) (5) and single-locus DNA sequencing of the repeat region of the Staphylococcus protein A gene (spa) (6) were performed for three representative MRSA isolates from each MRSA-positive store.

Prevalence of S. aureus and MRSA. Table 1 lists the prevalence of S. aureus and MRSA strains in the 120 meat samples examined. We recovered 121 S. aureus isolates from 47 meat samples. A total of 99 isolates (derived from 43 samples) were methicillin-susceptible S. aureus, and 22 isolates (from 6 samples) were MRSA. The overall prevalences of S. aureus were 45.6% in pork and 20% in beef, whereas MRSA was found in five (5.6%) pork samples and one (3.3%) beef sample. Two pork samples containing MRSA also yielded methicillin-susceptible S. aureus strains that lacked mecA. The use of BP medium supplemented with cefoxitin facilitated MRSA isola-
tion; however, this medium missed the recovery of MRSA from pork in three instances (Table 1). This demonstrates the importance of including an antibiotic-free medium even when screening for MRSA only.

The majority (73.3% [22/30]) of grocery stores surveyed had *S. aureus*-contaminated meats, and 10% (stores 4, 5, and 11) sold MRSA-positive meats. Noticeably, all four meat samples (three pork samples and one beef sample) from store 11 (belonging to supermarket chain A) were positive for MRSA only. The two remaining MRSA-positive pork samples were obtained from stores 4 and 5, belonging to chain B (Table 2). In addition, MRSA-positive meats from store 11 were collected on a different sampling date from those from stores 4 and 5. It is interesting to note that five out of six MRSA-positive meat samples (all were pork) were chain-branded meats (i.e., no other specific meat brands were shown on the label).

**Characteristics of MRSA isolates.** Major genotypic and phenotypic characteristics of representative MRSA isolates are summarized in Table 2. Among 22 confirmed MRSA isolates, 3 isolates recovered from two pork samples in stores 4 and 5 were positive for PVL, a two-component staphylococcal membrane toxin that targets leukocytes (Table 2). Primarily associated with CA-MRSA but not HA-MRSA, PVL is considered the principal virulence factor responsible for the spread of CA-MRSA in skin and soft-tissue infections (4). SCCmec typing identified two structure types among the 22 MRSA isolates, type IVa (a type IV subtype) for isolates from stores 4 and 5, and type II for all 19 isolates from store 11 (Table 2). SCCmec type IV is predominately associated with CA-MRSA. It is characterized by its smaller size (20 to 24 kb) and carriage of a limited number of antimicrobial resistance genes (22). Type II is larger (52 kb) and contains additional resistance genes (22). Susceptibility testing revealed that isolates belonging to the two SCCmec types differed in the numbers of antibiotics to which they were resistant (Table 2).

**PFGE using SmaI digestion identified two strain types (Table 2).** All 19 MRSA isolates from store 11 were determined to be the common HA-MRSA clone USA100 (14, 15). The three isolates from stores 4 and 5 belonged to the common CA-MRSA clone PFGE type USA300 (15). Additionally, isolates belonging to one clone also possessed the same phenotypic and genotypic characteristics as discussed above (Table 2). Based on this evidence, we analyzed three representative MRSA isolates, one from each MRSA-positive store, by the DNA sequencing methods *spa* typing and MLST. Two *spa* types (t002 and t008) and two multilocus sequence types (ST5 and ST8) were identified, and each was associated with one MRSA clone (Table 2). Previously identified MRSA clones related to pig farming were nontypeable by SmaI PFGE and harbored several closely related *spa* types (t011, t034, t108, t567, and t1254), all corresponding to ST398 (17, 18). This provided additional evidence that humans, not animals, are the likely contamination source for the two MRSA clones identified in this study.

**Summary.** To our knowledge, this is the first survey in the United States examining the prevalence and characteristics of MRSA in retail meats. Six samples (5%) contained MRSA strains, which were determined to be of two unique human epidemic clones, USA100 and USA300. Additionally, nearly 40% of the meats examined contained *S. aureus*, which was comparable to the prevalence rate recently reported in The Netherlands (19).

The high prevalence of *S. aureus* and the isolation of human

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**TABLE 1. Prevalence of *S. aureus* and MRSA in retail pork and beef samples in Baton Rouge, LA, in 2008**

<table>
<thead>
<tr>
<th>Meat type</th>
<th>No. of samples</th>
<th><em>S. aureus</em> including MRSAa</th>
<th>MRSA aloneb</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of isolates</td>
<td>No. (%) of positive samples</td>
<td>No. of isolates</td>
<td>No. (%) of positive samples</td>
</tr>
<tr>
<td>Pork</td>
<td>90</td>
<td>104 (45.6)</td>
<td>12</td>
<td>5 (5.6)</td>
</tr>
<tr>
<td>Beef</td>
<td>30</td>
<td>17 (60)</td>
<td>2</td>
<td>1 (3.3)</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>121 (39.2)</td>
<td>14</td>
<td>6 (5)</td>
</tr>
</tbody>
</table>

* Five out of seven supermarket chains were positive for *S. aureus*.
* MRSA strains were isolated from three grocery stores belonging to supermarket chains A and B.

**TABLE 2. Characteristics of two MRSA clones recovered from six retail meats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result for PFGE typec</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>USA100</td>
</tr>
<tr>
<td>Source</td>
<td>Supermarket chain</td>
</tr>
<tr>
<td>Store no.</td>
<td>11</td>
</tr>
<tr>
<td>Meat type</td>
<td>Pork and beef</td>
</tr>
<tr>
<td>Genotypic and phenotypic characteristics</td>
<td>Panton-Valentine leucocidin Negative</td>
</tr>
<tr>
<td>SCCmec type</td>
<td>II</td>
</tr>
<tr>
<td>Multilocus sequence type</td>
<td>ST-5</td>
</tr>
<tr>
<td><em>spa</em> type</td>
<td>t002</td>
</tr>
<tr>
<td>Antimicrobial susceptibility profiled</td>
<td>Chl' Cip' Cip' Dox'</td>
</tr>
<tr>
<td></td>
<td>Ery' Fus' Gen' Oxa' Ox' Rif' Van'</td>
</tr>
<tr>
<td></td>
<td>Kan' Oxa' Tet' Dox' Fus' Rif' Van'</td>
</tr>
<tr>
<td></td>
<td>Rif' Van'</td>
</tr>
</tbody>
</table>

* PFGE types were assigned based on two previous studies (14, 15).
* Antimicrobial agents are abbreviated as follows: Chl, chloramphenicol; Cip, ciprofloxacin; Cli, clindamycin; Dox, doxycycline; Ery, erythromycin; Fus, fusidic acid; Gen, gentamicin; Kan, kanamycin; Oxa, oxacillin; Rif, rifampin; Tet, tetracycline; and Van, vancomycin. In parentheses indicates intermediate resistance.
epidemic MRSA clones in retail meats raise public health concerns. Although *S. aureus* is generally regarded as an agent causing food-borne intoxication due to the production of heat-stable enterotoxins, the presence of MRSA in meats may pose a potential threat of infection to individuals who handle the food. Klyutmans et al. (11) reported a severe MRSA outbreak in 1995, which was most likely initiated by a contaminated food item. Additionally, a CA-MRSA strain has been implicated in a food-borne outbreak, although toxin production was the primary cause of illness (7). Therefore, great attention needs to be taken to prevent the introduction of MRSA from human carriers onto the meats they handle and thereby spreading the pathogen.

Our study indicates that MRSA, although at a low rate, is present in the U.S. food chain, likely due to human contamination. No “zoonotic” MRSA clones were found in the study. However, the study was limited in geographical region, survey period, and sample size. Further studies at the farm and retail levels involving larger sample sizes over time are needed to better assess the presence of MRSA in raw meats and the risk to meat handlers and consumers.

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REFERENCES


