Research Note

Characterization of Borderline Oxacillin-Resistant Staphylococcus aureus Isolated from Food of Animal Origin

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ABSTRACT

In this study, the molecular characteristics of food-derived oxacillin-resistant Staphylococcus aureus were determined. Eight borderline oxacillin-resistant strains with MICs of 2 to 4 μg/ml were identified from 132 S. aureus isolates of food origin. One of the two isolates with a MIC of 4 μg/ml was methicillin-resistant determinant (mecA) gene positive, and the other six with MICs of 2 μg/ml were mecA negative. The mecA-positive isolate was classified assequence type (ST)228, staphylococcal protein A (spa) type t041, and carried the staphylococcal cassette chromosome mec type I element. Two borderline oxacillin-resistant strains were classified as spa t008 and ST8, and the remaining five as spa t164 and ST20. The mecA-positive strain and four borderline oxacillin-resistant strains were found enterotoxigenic. The enterotoxin genes detected in these strains included selp, egc1, and sed-sej-serl. The borderline-resistant S. aureus isolates from a manually handled product, i.e., minced pork, were shown genetically related to strains associated with human infections. This suggests that humans can be considered as a source of contamination of this food with oxacillin-resistant S. aureus strains. The genotypes of the investigated milk borderline-resistant isolates were shown to occur not only in cows, but also in humans. Since manual handling is reduced in raw milk production, a human origin of S. aureus seems unlikely. Because knowledge of the genotypes of animal staphylococci is limited, more research is needed to address the question of the origin of antibiotic-resistant S. aureus strains in food.

Many human isolates of Staphylococcus aureus areresistant to β-lactam antibiotics. Strains showing this phenotype are methicillin (oxacillin)-resistant S. aureus (MRSA). Resistance to methicillin is chromosomally encoded by the methicillin-resistant determinant (mecA) gene, located on a mobile genetic element called the staphylococcal chromosomal cassette (SCC). S. aureus strains harboring the mecA gene are resistant to all known β-lactam antibiotics (1). The MICs for oxacillin (which replaced the no longer clinically used methicillin) for MRSA range from 0.5 to more than 1,000 μg/ml (9).

Another type of oxacillin resistance is called borderline oxacillin resistance. This phenotype has been described in S. aureus isolates that do not carry the mecA gene, but are characterized by a certain level of oxacillin resistance. The MICs for borderline oxacillin resistance were determined to be 1 to 8 μg/ml (1). Two mechanisms have been proposed to explain this type of resistance, the hyperproduction of β-lactamase and modified resistance due to the production of penicillin-binding protein 4 with reduced affinity for oxacillin (10, 17).

Food products were recently described as a source of isolates’ participation in human infections have not been investigated thoroughly (13, 14). The aim of this study was to characterize borderline oxacillin-resistant S. aureus strains isolated from food products of animal origin.

MATERIALS AND METHODS

Bacterial strains. One hundred thirty-two S. aureus strains were obtained from food products of animal origin. Seventy-three isolates were derived from 460 samples of raw quarter milk from cows without clinical symptoms of mastitis. About 40 samples of milk were taken every 2 months during a 2-year period from six herds located in Lower Silesia (Poland). Forty-three isolates were from 76 raw minced porcine, 23 bovine, and 63 turkey meat samples; six strains from 24 samples of Polish “white” sausage; eight from 69 cakes with sour cream; and two from 18 samples of potato salad with mayonnaise. The food products were purchased from a retail market. Only one isolate per food product or milk sample was used in the investigation. Reference MRSA strains representing diverse SCC mec (SCCmec), spa (encoding staphylococcal protein A), and ST (sequence type) were kindly provided by Prof. Waleria Hryniewicz of the National Medicines Institute Warsaw, Poland. Enterotoxigenic reference strains were kindly provided by Dr. Gerard Lina of the Centre National de Référence des Toxémes Staphylococciques, Faculté de Médecine, Lyon, France. The isolates were identified as S. aureus based on their biochemical properties (Api-STAPH, bioMérieux, Inc., Marcy l’Etoile, France) and their ability to coagulate rabbit plasma and produce a clumping factor. In addition, all strains were PCR
TABLE 1. Oxacillin resistance, meca gene presence, genotype, and enterotoxin gene content of food-derived Staphylococcus aureus isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Origin</th>
<th>spa type</th>
<th>ST</th>
<th>Enterotoxin gene(s)</th>
<th>meca gene</th>
<th>Oxacillin MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Raw porcine minced meat</td>
<td>t041</td>
<td>228</td>
<td>selp</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Raw porcine minced meat</td>
<td>t008</td>
<td>8</td>
<td>sed, sej, selr</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Raw porcine minced meat</td>
<td>t008</td>
<td>8</td>
<td></td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Raw milk</td>
<td>t164</td>
<td>20</td>
<td>egcl, egcl</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Raw milk</td>
<td>t164</td>
<td>20</td>
<td></td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Raw milk</td>
<td>t164</td>
<td>20</td>
<td>egcl</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>Raw milk</td>
<td>t164</td>
<td>20</td>
<td>egcl</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>8</td>
<td>Raw milk</td>
<td>t164</td>
<td>20</td>
<td></td>
<td>-</td>
<td>2</td>
</tr>
</tbody>
</table>

screened with primers for the S. aureus–specific nuc gene, which encodes a thermonuclease, as described by Martin et al. (8).

Preparation of bacterial DNA. Bacterial DNA was prepared as described by Lis et al. (7).

Antimicrobial susceptibility testing. All S. aureus strains were tested for their susceptibility to oxacillin on Mueller-Hinton agar (bioMérieux, Inc.) containing 6 µg/ml oxacillin with 4% NaCl. The results were recorded after 24 h of incubation at 35°C and interpreted according to the manufacturer’s instructions. The MICs for oxacillin were determined with the E-test (AB Biodisk, Solna, Sweden).

meca gene detection and determination of SCCmec type. The presence of the meca gene and the determination of SCCmec type were performed with the primers described by Milheiro et al. (11). The PCR products were electrophoretically resolved in 1.5% agarose gel containing 0.5 µg/ml ethidium bromide and then photographed with the GelDocXR System (Bio-Rad, Hercules, CA).

Determination of spa type. The sequence of the repeat-containing region of the spa gene was obtained from both strands of the PCR product, according to Harmsen et al. (5). Sequencing was performed with the BigDye Terminator ready-reaction cycle sequencing kit (Applied Biosystems, Foster City, CA). The analysis of repeats and the assignment of spa type were performed with the resources of the Ridom SpaServer (http://spa.ridom.de).

Multilocus sequence typing. The ST of selected S. aureus strains were determined with the method of Enright et al. (4). The sequences obtained from both strands of the PCR product were analyzed with BioEdit software (http://mbio.ncsu.edu/BioEdit/bioedit.html), and further assignment of the ST was performed with a platform found at: http://www.mlst.net.

Detection of enterotoxin genes. The detection of sea, see, seg, seh, sei, selj, selk, sell, selm, seln, selo, selp, selu, and tst genes was performed as described previously (7).

RESULTS

Oxacillin resistance and meca gene presence in S. aureus strains. Of the 132 food-derived S. aureus strains, 8 (6%) demonstrated borderline oxacillin resistance. Seven (5.3%) oxacillin-resistant S. aureus were meca negative. Two of these strains were isolated from porcine minced meat and 5 from raw cow’s milk. The range of oxacillin MICs for these cultures was between 2 and 4 µg/ml. One strain (0.7%) from minced pork possessed the meca gene. The oxacillin MIC for the meca-positive isolate was 4 µg/ml (Table 1).

Molecular characteristics of the oxacillin-resistant S. aureus strains. The meca-positive S. aureus isolated from porcine meat was classified as ST228, spa type t041, and it harbored the SCCmec type I. This strain was found to bear the enterotoxin-like selp gene. The genotypes of the two borderline oxacillin-resistant strains from porcine raw meat were determined to be t008, ST8. One of these strains carried the sed-sej-selr enterotoxin genes. The genotypes of five of the borderline oxacillin-resistant strains from cow’s milk were determined to be t164, ST20, and three of them carried the egcl enterotoxin gene (Table 1).

DISCUSSION

Borderline oxacillin-resistant S. aureus strains have been detected in food (14, 15), but their molecular characteristics are largely unknown. Although the incidence of borderline oxacillin-resistant S. aureus strains among human clinical isolates was reported to be only about 5%, they have been associated with community-acquired and hospital infections (6).

In the current study, eight S. aureus strains showed borderline oxacillin resistance. The MIC of oxacillin for these isolates was 2 µg/ml, except for two strains, one with the meca gene and the other meca negative, which showed higher resistance (MIC of 4 µg/ml). The meca-negative isolate had a MIC typical for borderline oxacillin-resistant isolates (1). The low MIC value of the meca-positive isolate may indicate a heteroresistant phenotype (9). All five milk-derived borderline oxacillin-resistant strains were classified to ST20 and spa type t164. In a report by Monecke et al. (12), two ST20 S. aureus clones were found in cattle. Two borderline oxacillin-resistant strains from porcine minced meat were assigned to t008, ST8. ST8 belongs to the major clonal complex CC8. In the United States, a specific ST8, spa t008, clone called USA300 was shown responsible for the majority of community-acquired infections causing major public health problems. The occurrence of this clone has been reported in several European countries (16). The meca-positive S. aureus strain from porcine meat identified in our study yielded ST228 SCCmec I, spa type t041, and carried a type I SCCmec cassette. This suggests a human origin, as ST228 belongs to one of the major staphylococcal
clonal complexes, CC5, which contributes to a significant part of infections in hospital settings worldwide (2). Previously examined MRSA clones related to pigs harbored several spa types (t011, t108, t567, and t1939), but all were assigned to ST398 (18). This suggests that humans, not animals, were the contamination source for the mecA-positive strain from porcine meat identified in our study, illustrating the possibility of transmission of typically hospital-derived S. aureus strains to food.

A direct link between S. aureus antibiotic resistance and enterotoxigenicity has been established, as these staphylococcal strains have been considered a causative agent of antibiotic-associated diarrhea. The proposed mechanism of antibiotic-associated diarrhea development includes the alteration of resident gut flora, caused by antibiotic therapy, which eventually leads to the expression of pathogenic (enterotoxigenic) properties of intestinal staphylococci (3). Enterotoxin genes found in five of our borderline oxacillin-resistant S. aureus strains, including the mecA-positive strain, demonstrate that food can be considered a potential vector of strains implicated in antibiotic-associated diarrhea development.

The borderline-resistant S. aureus isolates from the manually handled product, i.e., minced pork, were shown genetically related to strains associated with human infections. This suggests that humans can be considered a source of contamination of this food with oxacillin-resistant S. aureus strains. The genotypes of the investigated milk borderline-resistant isolates were shown to occur not only in cows, but also in humans. Since manual handling is reduced in raw milk production, a human origin of S. aureus seems unlikely. Knowledge of the genotypes of animal staphylococci is limited; thus, more research is needed to address the question of the origin of antibiotic-resistant S. aureus strains in food.

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REFERENCES