Research Note

Retail Ready-to-Eat Food as a Potential Vehicle for Staphylococcus spp. Harboring Antibiotic Resistance Genes

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MS 13-466: Received 25 October 2013/Accepted 20 December 2013

ABSTRACT

Ready-to-eat (RTE) food, which does not need thermal processing before consumption, could be a vehicle for the spread of antibiotic-resistant microorganisms. As part of general microbiological safety checks, staphylococci are routinely enumerated in these kinds of foods. However, the presence of antibiotic-resistant staphylococci in RTE food is not routinely investigated, and data are only available from a small number of studies. The present study evaluated the phenotypic and genotypic antibiotic resistance profile of Staphylococcus spp. isolated from 858 RTE foods (cheeses, cured meats, sausages, smoked fishes, salads). Of 113 strains isolated, S. aureus was the most prevalent species, followed by S. xylosus, S. saprophyticus, and S. epidermidis. More than half (54.9%) of the isolates were resistant to at least one class of tested antibiotic; of these, 35.4% of the strains were classified as multidrug resistant. Most of the isolates were resistant to cefoxitin (49.6%), followed by clindamycin (39.3%), tigecycline (27.4%), quinupristin-dalfopristin (22.2%), rifampin (20.5%), tetracycline (17.9%), and erythromycin (8.5%). All methicillin-resistant staphylococci harbored the mecA gene. Among the isolates resistant to at least one antibiotic, 38 harbored tetracycline resistance determinant tet(M), 24 harbored tet(L), and 9 harbored tet(K). Of the isolates positive for tet(M) genes, 34.2% were positive for the Tn916-Tn1545–like integrase family gene. Our results indicated that retail RTE food could be considered an important route for the transmission of antibiotic-resistant bacteria harboring multiple antibiotic resistance genes.

Staphylococcus spp. are ubiquitous gram-positive bacteria that represent part of the normal bacterial microflora of the skin and mucosal surfaces of humans and animals. Some Staphylococcus spp., especially S. aureus, are the leading causes of nosocomial infections (31). On the other hand, some species of coagulase-negative staphylococci, such as S. xylosus and S. carnosus, are considered technologically important in the manufacturing processes of meat products. They are used as starters in dry fermented sausages to ensure the quality and safety of the final product (1). Their favorable activities are expressed in color and aroma development and in proteolytic and lipolytic succession, which all contribute to the sensory properties of the final product (33).

In recent years, the spread of antibiotic resistance in bacteria, including staphylococci, has been increasing and may represent a hazard to human health. The widespread application of antimicrobials in medical and veterinary practice, the use of antibiotics in agriculture (such as avoparcin or tetracyclines), and the common use of antiseptics and disinfectants have resulted in selective pressure (18, 22). One of the resistance acquisitions is mediated by horizontal gene transfer of mobile genetic elements carrying antibiotic resistance genes. Bacteria containing transmissible antibiotic resistance elements, like plasmids, integrons, and pathogenicity islands, are a risk for public health because they can act as a reservoir for spreading resistance genes (14, 24). Consequently, food-associated bacteria harboring such transferable antibiotic resistance genes are of major concern. Therefore, a question arises regarding the contribution of food as a reservoir for the spread of antibiotic resistance.

For a long time, the focus has been on staphylococci associated with the hospital environment; however, accumulating evidence about antibiotic resistance and the mechanisms of its transmission has made researchers examine its epidemiology from a broader perspective. The interest in the antibiotic resistance of bacteria isolated from food has focused on studies of pathogens such as Escherichia coli O157:H7 (7), Campylobacter spp. (13), Salmonella (29), Clostridium spp. (27), and Listeria monocytogenes (10). There has been increased interest in the role of Staphylococcus spp. isolated from food in the transmission of antibiotic resistance, with research focused mainly on strains isolated from raw food, especially milk and meat. Such food is usually subjected to further treatment, for example, thermal treatment such as boiling or baking. It seems more important for a consumer to examine food that will be consumed directly. Staphylococci are routinely enumerated in a wide variety of ready-to-eat (RTE) foods, as part of general microbiological safety...
checks. However, the presence of antibiotic-resistant staphylococci in food is not routinely investigated.

Because RTE food does not need thermal processing before consumption, it could serve as a vector for the spread of antibiotic-resistant microorganisms. Thus, the objective of this work was to evaluate the occurrence of antibiotic-resistant staphylococci from RTE food and the presence of genes that encode resistance to antibiotics to which most of the strains demonstrated phenotypic resistance.

**MATERIALS AND METHODS**

**Isolation of staphylococci.** Staphylococci were isolated from 858 samples obtained from various retail RTE products (cheeses, cured meats, sausages, smoked fishes, salads) obtained from local markets in Olsztyn, Poland. Food samples (10 g) were homogenized in 90 ml of buffered peptone water (Merck, Darmstadt, Germany), incubated overnight at 37°C, and streaked on selective plates containing mannitol salt phenol red agar (Merck). Mannitol-positive colonies were differentiated as coagulase positive and coagulase negative with a test to detect the production of a clumping factor (Staphylase test kit, Oxoid, Basingstoke, UK) and production of coagulase on rabbit plasma fibrinogen medium (bioMérieux, Marcy l’Etoile, France). Gram-positive and catalase-positive bacteria were presumptively identified as staphylococci and were selected for further biochemical identification. The staphylococcal isolates were deposited in the laboratory culture collection. Prior to analysis, isolates were stored with the Microbank system at −80°C (Biocorp, Warsaw, Poland).

**Bacterial strains and DNA extraction.** For DNA extraction, bacterial strains were streaked onto brain heart infusion agar (Merck) and incubated overnight at 37°C. The colonies were suspended in Tris-EDTA buffer and were lysed by lysozyme enzyme (0.6 mg/ml) from *S. simulans* (A&A Biotechnology, Gdynia, Poland). The total genomic DNA of isolated and reference strains was extracted using the Genomic Mini DNA purification kit (A&A Biotechnology) according to the manufacturer’s instructions.

**Molecular identification of isolates to the genus level.** PCR was used to confirm the affiliation to the *Staphylococcus* genus; multiplex PCR was used to identify four species at once (*S. aureus*, *S. xylosus*, *S. saprophyticus*, and *S. epidermidis*), according to the method of Morot-Bizot et al. (19), with minor modifications. The PCR reaction mixture of 25 µl contained 0.5 µM (each) primer, 200 µM (each) deoxyribonucleoside triphosphate (Thermo Scientific Fermentas, St. Leon-Rot, Germany), 3 mM MgCl₂ (Thermo Scientific Fermentas), 1 U of Taq DNA polymerase in 1 × Green Buffer (Thermo Scientific Fermentas), and 3 µl of DNA template. Amplification was carried out in an MJ Mini 48-well personal thermal cycler (Bio-Rad, Hercules, CA); a polymerase activation step (95°C for 15 min) was followed by 35 cycles of denaturation (95°C for 1 min, 30 s), annealing (55°C for 1 min), and extension (72°C for 1 min), with a final extension step (72°C for 7 min). The PCR reaction mixture was analyzed by electrophoresis through a 2% high-resolution agarose gel (Promega, Madison, WI) in 1 × Tris-borate-EDTA buffer (pH 8.3). The sizes of the amplification products were estimated by comparison with a 100-bp molecular size ladder (Thermo Scientific Fermentas). Gels were stained with ethidium bromide and viewed under UV light using a transilluminator (BXT-26.M, Uvitec, Cambridge, UK). Each profile was visually compared with those obtained from the staphylococcal reference strains: *S. aureus* ATCC 43300, *S. xylosus* ATCC 29971, *S. saprophyticus* ATCC 49453, and *S. epidermidis* ATCC 49461.

**Antimicrobial susceptibility test.** Antimicrobial susceptibility testing was performed on Mueller-Hinton agar (bioMérieux), using the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) document M100-S20 (5). Isolates were tested for their susceptibility to erythromycin (E, 15 µg), clindamycin (DA, 2 µg), gentamicin (CN, 120 µg), ceftoxin (FOX, 30 µg), norfloxacin (NOR, 10 µg), ciprofloxacin (CIP, 5 µg), tetracycline (TE, 30 µg), rifampin (RD, 5 µg), nitrofurantoin (F, 300 µg), linezolid (LZD, 30 µg), chloramphenicol (C, 30 µg), trimethoprim (W, 5 µg), trimethoprim-sulfamethoxazole (SXT, 25 µg), quinupristin-dalfopristin (QDA, 15 µg), and tigecycline (TGC, 1 µg) using antimicrobial disks (Oxoid). Plates were incubated at 37°C for 20 to 24 h, and the results were interpreted according to CLSI document M100-S20 (5).

**PCR detection of antimicrobial resistance genes.** All strains that were phenotypically resistant to at least one antibiotic tested were examined for the presence of the resistance genes. PCR for detection of *mecA* genes (533 bp) was performed with primers and conditions previously described (2), with minor modifications. PCR cycles consisted of 40 cycles of denaturation at 96°C for 60 s, annealing at 55°C for 30 s, and extension at 72°C for 90 s, with a final extension of 72°C for 5 min. All isolates were examined for the presence of the genes encoding efflux proteins (tet(K) and tet(L)) and genes encoding ribosomal protection proteins (tet(M)). For all the tet(M)-positive isolates, the presence of conjugative transposons of the Tn916-Tn545 family was determined by using primers targeting the integrase gene *int* according to Doherty et al. (6). TE efflux gene *tet(K)* amplification was performed according to Gevers et al. (8), with an initial denaturation of 94°C for 5 min followed by 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, with a final extension of 72°C for 10 min. PCR detection of resistance to the TE genes tet(M) and tet(L) and the macrolide *erm(B)* was determined using the specific primers and the conditions reported by Rizzotti et al. (23). Amplification of *erm(B)*, *tet(M)*, and *tet(L)* was performed in multiplex (triplex) PCR. The amplicons were evaluated by 1.5% agarose gel electrophoresis followed by staining in ethidium bromide (0.5 mg/ml) and were visualized on a UV transilluminator. In all PCR's determining antimicrobial resistance genes, strains from the Department of Industrial and Food Microbiology (University of Warmia and Mazury, Olsztyn, Poland) were used as positive controls: *E. faecalis* 20138EK (positive for tet(M), tet(L), erm(B), and int) and *E. faecalis* 15555EK (positive for tet(K)).

**RESULTS**

Staphylococci strains (*n* = 113) isolated from different RTE foods were used in this study. According to PCR amplification, 55 isolates were identified as *S. aureus*, 23 as *S. xylosus*, 6 as *S. saprophyticus*, 4 as *S. epidermidis*, and 25 as other *Staphylococcus* spp. All staphylococcal isolates were examined for their susceptibility to 15 antibiotics. The data obtained from the disc diffusion testing are summarized in Table 1. The overall percentages of antimicrobial-resistant isolates were FOX (49.6%), DA (39.3%), TGC (27.4%), QDA (22.2%), RD (20.5%), TE (17.9%), and E (8.5%). Resistance to other antibiotics tested ranged from 7.7 to 1.7%. More than half of all investigated isolates (62 [54.9%] of 113) were resistant to at least one class of antibiotic, and 40 of these strains were classified as multidrug resistant (MDR; resistant to three or more classes of antibiotics). Most MDR strains (*n* = 13) revealed
TABLE 1. Antibiotic resistance of Staphylococcus spp. isolated from RTE food*

<table>
<thead>
<tr>
<th>Antibiotic symbol</th>
<th>Resistance (%)</th>
<th>Susceptibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOX</td>
<td>56 (49.6)</td>
<td>57 (50.4)</td>
</tr>
<tr>
<td>DA</td>
<td>46 (39.3)</td>
<td>67 (59.3)</td>
</tr>
<tr>
<td>TGC</td>
<td>32 (27.4)</td>
<td>81 (71.7)</td>
</tr>
<tr>
<td>QDA</td>
<td>26 (22.2)</td>
<td>87 (77)</td>
</tr>
<tr>
<td>RD</td>
<td>24 (20.5)</td>
<td>89 (78.8)</td>
</tr>
<tr>
<td>TE</td>
<td>21 (17.9)</td>
<td>92 (81.4)</td>
</tr>
<tr>
<td>CN</td>
<td>20 (17.1)</td>
<td>93 (82.3)</td>
</tr>
<tr>
<td>E</td>
<td>10 (8.5)</td>
<td>103 (91.2)</td>
</tr>
<tr>
<td>W</td>
<td>9 (7.7)</td>
<td>104 (92)</td>
</tr>
<tr>
<td>LZD</td>
<td>7 (6.0)</td>
<td>106 (93.8)</td>
</tr>
<tr>
<td>F</td>
<td>6 (5.1)</td>
<td>107 (94.7)</td>
</tr>
<tr>
<td>SXT</td>
<td>6 (5.1)</td>
<td>107 (94.2)</td>
</tr>
<tr>
<td>CIP</td>
<td>4 (3.4)</td>
<td>109 (96.5)</td>
</tr>
<tr>
<td>C</td>
<td>3 (2.6)</td>
<td>110 (97.3)</td>
</tr>
<tr>
<td>NOR</td>
<td>2 (1.7)</td>
<td>111 (98.2)</td>
</tr>
</tbody>
</table>

a n = 113. FOX, cefoxitin; DA, clindamycin; TGC, tigecycline; QDA, quinupristin-dalfopristin; RD, rifampin; TE, tetracycline; CN, gentamicin; E, erythromycin; W, trimethoprim; LZD, linezolid; F, nitrofurantoin; SXT, trimethoprim-sulfamethoxazole; CIP, ciprofloxacin; C, chloramphenicol; NOR, norfloxacin.

Simultaneous resistance to six classes of antibiotics, followed by 11 strains to four classes, 9 strains to five classes, and 4 strains to three classes. Two of the examined strains (S. xylosus 852G and S. xylosus 629G) revealed simultaneous resistance to antibiotics of eight various classes, and one strain (S. aureus 15G) was resistant to seven classes of antibiotics (Table 2). All MDR strains (100%) were resistant to FOX, and the majority were resistant to DA (87.5%) and TGC (75%).

All of the strains resistant to at least one antibiotic were screened to detect resistance genes by PCR. The mecA gene, which lies in the SCCmec resistance island, was carried by 52 of the 62 analyzed isolates. All of the mecA-positive isolates display a phenotype of methicillin (oxacillin) resistance. All MDR S. aureus, S. epidermidis, and S. xylosus isolates harbored the mecA gene.

The majority of investigated isolates (n = 38) harbored TE resistance determinant tet(M). The tet(L) genes also appeared but were less common (n = 24) and were observed mostly in S. aureus. Nine of the strains harbored the tet(K) gene. In S. aureus, the isolated tet(K) gene was always associated with tet(M) and tet(L), but in S. xylosus and Staphylococcus spp. isolates, this gene was detected either alone or associated with the tet(M) gene. The strains that harbored both tet(M) and tet(L) genes (n = 22) were largely isolated from cured meat; only one strain was isolated from cheese.

Of those isolates positive for tet(M) genes (38 of 62), 13 were positive for the Tn916-Tn1545–like integrase family gene. Thirteen isolates that were phenotypically TE susceptible were found to carry at least one of the tet genes. On the contrary, three staphylococcal isolates were phenotypic resistant to TE but negative for the tet(M), tet(L), or tet(K) gene. Of the 10 staphylococci strains resistant to E, all were negative for the tested erm(B) gene.

DISCUSSION

In recent years, systematic growth in the number of antibiotic-resistant strains in the human environment has been observed. Most of the previous studies on resistance have concentrated on staphylococci isolated from clinical samples (26, 31). Recently, some researchers have suggested that food could be an appropriate environment for resistant and multiresistant strains and that the food chain could play a key role in the transmission of resistance between the environment and humans (3, 12, 20, 22). This study gives a better understanding of the multiple antimicrobial resistance profiles observed in staphylococcal isolates from RTE food in Poland.

The most prevalent species identified in RTE food in our study were S. aureus and S. xylosus, which agrees with results reported by other European researchers (21, 25). In the tests performed, resistance to at least one antibiotic was observed in 54.9% of the investigated strains. The recovery of high percentages of MDR staphylococci from RTE food suggests that they may be an important reservoir of antimicrobial resistance phenotypes. Most isolates revealed a resistance to FOX and carry the mecA gene; in the case of strains isolated from RTE food, this is an alarming phenomenon because it determines methicillin-resistant strains that are phenotypically resistant to all β-lactam antibiotics used so far in treatment, namely, penicillins, aminopenicillins, isoxazolyl penicillins (oxacillin, cloxacillin, dicloxacillin, flucloxacillin), nafcillin, cephalosporins, penicillins with inhibitors, cephalosporins with inhibitors, and carbapenems (32). Our analysis of the results revealed another interesting relationship, namely, that up to 80.8% of methicillin-resistant strains showed, at the same time, resistance to DA. A disturbing fact is that most of our isolates were also resistant to TGC, a new glycolcycline broad-spectrum antibiotic.

In other recently reported studies, lower isolation frequencies for methicillin-resistant S. aureus (MRSA) in foods were generally found. In an Italian survey of 1,634 foodstuff samples, 6 (0.4%) MRSA strains were isolated from bovine milk and cheese (20). In Japan, Hammad et al. (12) examined 200 samples of retail RTE raw fish (sashimi), from which they recovered 10 MRSA or methicillin-resistant coagulase-negative staphylococci (MR-CoNS) isolated from 10 (5%) different samples. From 70 samples of RTE food of animal origin examined by Podkowik et al. (21), 15 (36%) of 67 strains of coagulase-negative staphylococci harbored the mecA gene, and none of the S. aureus isolates characterized in the work were mecA positive. We got slightly different results, which may be due to a much larger number of samples tested. We examined 858 samples, of which 6% were positive for mecA (35 MRSA and 17 MR-CoNS strains) (Table 2). All MRSA and MR-CoNS carry the mecA gene, in contrast to previously published results (12, 17).
TABLE 2. Antimicrobial resistance phenotypes and resistance genes identified in staphylococcal strains phenotypically resistant to at least one antibiotic

<table>
<thead>
<tr>
<th>Staphylococcus isolates</th>
<th>Gene mecA</th>
<th>Tetracycline resistance genes</th>
<th>Tn916/Tn545 Phenytopic resistance</th>
<th>Food source</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus 804G</td>
<td>—</td>
<td>tet(L) tet(M)</td>
<td>NC</td>
<td>Smoked fish</td>
</tr>
<tr>
<td>S. aureus 87G</td>
<td>mec(A)</td>
<td>tet(L) tet(M)</td>
<td>E</td>
<td>Cured meat</td>
</tr>
<tr>
<td>S. aureus 111.7G</td>
<td>mec(A)</td>
<td>tet(L) tet(M)</td>
<td>int</td>
<td>Cured meat</td>
</tr>
<tr>
<td>S. aureus 486mG</td>
<td>mec(A)</td>
<td>NC</td>
<td>FOX</td>
<td>Cured meat</td>
</tr>
<tr>
<td>S. aureus 111.7G</td>
<td>mec(A)</td>
<td>tet(M)</td>
<td>FOX</td>
<td>Cured meat</td>
</tr>
<tr>
<td>S. aureus 568mG</td>
<td>mec(A)</td>
<td>tet(M)</td>
<td>DA, FOX</td>
<td>Sausage</td>
</tr>
<tr>
<td>S. aureus 806G</td>
<td>mec(A)</td>
<td>NC</td>
<td>DA, FOX</td>
<td>Sausage</td>
</tr>
<tr>
<td>S. aureus 765G</td>
<td>mec(A)</td>
<td>NC</td>
<td>E, DA</td>
<td>Smoked fish</td>
</tr>
<tr>
<td>S. aureus 111.3G</td>
<td>—</td>
<td>tet(L) tet(M)</td>
<td>TGC, QD</td>
<td>Cured meat</td>
</tr>
<tr>
<td>S. aureus 630G</td>
<td>mec(A)</td>
<td>tet(M)</td>
<td>FOX, F, CN</td>
<td>Cheese</td>
</tr>
<tr>
<td>S. aureus 845G</td>
<td>mec(A)</td>
<td>NC</td>
<td>DA, FOX, F, CN</td>
<td>Vegetable salad</td>
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<tr>
<td>S. aureus 534G</td>
<td>mec(A)</td>
<td>NC</td>
<td>DA, FOX, TGC, RD</td>
<td>Smoked fish</td>
</tr>
<tr>
<td>S. aureus 618mG</td>
<td>mec(A)</td>
<td>tet(M)</td>
<td>DA, FOX, TE, QD</td>
<td>Cured meat</td>
</tr>
<tr>
<td>S. aureus 111.6bG</td>
<td>mec(A)</td>
<td>tet(L) tet(M)</td>
<td>DA, FOX, TE, CN, QD</td>
<td>Cured meat</td>
</tr>
<tr>
<td>S. aureus 27G</td>
<td>mec(A)</td>
<td>int</td>
<td>DA, FOX, TGC, RD, QD</td>
<td>Cured meat</td>
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<tr>
<td>S. aureus 177G</td>
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<td>int</td>
<td>DA, FOX, TGC, TE, QD</td>
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<tr>
<td>S. aureus 612bmG</td>
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<td>int</td>
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<tr>
<td>S. aureus 610amG</td>
<td>mec(A)</td>
<td>tet(M)</td>
<td>DA, FOX, W, TGC, SXT</td>
<td>Smoked fish</td>
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<tr>
<td>S. aureus 625G</td>
<td>mec(A)</td>
<td>tet(M)</td>
<td>FOX, F, W, TGC, SXT, RD</td>
<td>Cheese</td>
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<tr>
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<td>DA, FOX, TGC, RD, CN, LZD</td>
<td>Cheese</td>
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<tr>
<td>S. aureus 28G</td>
<td>mec(A)</td>
<td>tet(L) tet(M) tet(K)</td>
<td>DA, FOX, TGC, RD, CN, QD</td>
<td>Cured meat</td>
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<tr>
<td>S. aureus 623G</td>
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<td>NC</td>
<td>DA, FOX, TGC, RD, CN, QD</td>
<td>Cheese</td>
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<tr>
<td>S. aureus 26G</td>
<td>mec(A)</td>
<td>tet(L) tet(M) tet(K)</td>
<td>DA, FOX, TGC, SXT, RD, QD</td>
<td>Cured meat</td>
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<tr>
<td>S. aureus 115G</td>
<td>mec(A)</td>
<td>tet(L) tet(M) tet(K)</td>
<td>DA, FOX, TGC, TE, CN, QD</td>
<td>Cured meat</td>
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<tr>
<td>S. aureus 166G</td>
<td>mec(A)</td>
<td>NC</td>
<td>DA, FOX, TGC, TE, CN, QD</td>
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<tr>
<td>S. aureus 90G</td>
<td>mec(A)</td>
<td>tet(L) tet(M) tet(K)</td>
<td>DA, FOX, TGC, TE, RD, QD</td>
<td>Cured meat</td>
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<tr>
<td>S. aureus 105G</td>
<td>mec(A)</td>
<td>tet(L) tet(M) tet(K)</td>
<td>DA, FOX, TGC, TE, RD, QD</td>
<td>Cured meat</td>
</tr>
<tr>
<td>S. aureus 618bmG</td>
<td>mec(A)</td>
<td>tet(M)</td>
<td>DA, FOX, TGC, TE, RD, QD</td>
<td>Cured meat</td>
</tr>
<tr>
<td>S. aureus 23G</td>
<td>mec(A)</td>
<td>tet(M)</td>
<td>DA, FOX, TGC, CIP, RD, CN, QD</td>
<td>Cured meat</td>
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<tr>
<td>S. aureus 92G</td>
<td>mec(A)</td>
<td>int</td>
<td>DA, FOX, TGC, TE, RD, QD</td>
<td>Cured meat</td>
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<td>S. aureus 108G</td>
<td>mec(A)</td>
<td>int</td>
<td>DA, FOX, TGC, TE, RD, QD</td>
<td>Cured meat</td>
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<tr>
<td>S. aureus 116bG</td>
<td>mec(A)</td>
<td>int</td>
<td>DA, FOX, TGC, TE, RD, QD</td>
<td>Cured meat</td>
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<tr>
<td>S. aureus 98G</td>
<td>mec(A)</td>
<td>int</td>
<td>DA, FOX, W, TE, SXT, QD</td>
<td>Cured meat</td>
</tr>
<tr>
<td>S. aureus 22G</td>
<td>mec(A)</td>
<td>tet(M)</td>
<td>DA, FOX, W, TGC, SXT, RD, CN</td>
<td>Cured meat</td>
</tr>
<tr>
<td>S. aureus 578.2G</td>
<td>mec(A)</td>
<td>int</td>
<td>E, DA, FOX, NOR, W, TGC, RD</td>
<td>Cured meat</td>
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<tr>
<td>S. aureus 15G</td>
<td>mec(A)</td>
<td>tet(L) tet(M) tet(K)</td>
<td>DA, FOX, W, TGC, RD, CN, QD</td>
<td>Cured meat</td>
</tr>
<tr>
<td>S. epidermidis 111.6aG</td>
<td>—</td>
<td>tet(L) tet(M) tet(K)</td>
<td>C</td>
<td>Sailage</td>
</tr>
<tr>
<td>S. epidermidis 774GA</td>
<td>mec(A)</td>
<td>tet(M)</td>
<td>E, FOX, W</td>
<td>Cottage cheese</td>
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<td>S. epidermidis 849G</td>
<td>mec(A)</td>
<td>tet(M)</td>
<td>DA, FOX, LZD, QD</td>
<td>Poultry</td>
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<td>S. saprophyticus 390G</td>
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<td>tet(M)</td>
<td>E, DA, FOX, TE, CIP, CN</td>
<td>Cheese</td>
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<tr>
<td>S. xylosus 773G</td>
<td>—</td>
<td>tet(M)</td>
<td>E</td>
<td>Cheese</td>
</tr>
<tr>
<td>S. xylosus 269G</td>
<td>mec(A)</td>
<td>tet(M)</td>
<td>FOX</td>
<td>Cereal bar</td>
</tr>
<tr>
<td>S. xylosus 301G</td>
<td>mec(A)</td>
<td>tet(M)</td>
<td>FOX</td>
<td>Cheese</td>
</tr>
<tr>
<td>S. xylosus 766G</td>
<td>mec(A)</td>
<td>NC</td>
<td>TGC</td>
<td>Cured meat</td>
</tr>
<tr>
<td>S. xylosus 772G</td>
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<td>NC</td>
<td>DA, FOX</td>
<td>Cheese</td>
</tr>
<tr>
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<td>mec(A)</td>
<td>NC</td>
<td>DA, FOX</td>
<td>Cottage cheese</td>
</tr>
<tr>
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<td>NC</td>
<td>DA, FOX, NOR</td>
<td>Sausage</td>
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<td>NC</td>
<td>DA, FOX, TGC, TE, E</td>
<td>Sausage</td>
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<td>S. xylosus 614mG</td>
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<td>tet(M) tet(K)</td>
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<td>Smoked fish</td>
</tr>
<tr>
<td>S. xylosus 582G</td>
<td>mec(A)</td>
<td>NC</td>
<td>DA, FOX, TGC, RD, LZD</td>
<td>Sausage</td>
</tr>
<tr>
<td>S. xylosus 483mG</td>
<td>mec(A)</td>
<td>tet(L)</td>
<td>DA, FOX, TGC, RD, CN, LZD</td>
<td>Smoked fish</td>
</tr>
<tr>
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<td>mec(A)</td>
<td>tet(M)</td>
<td>E, DA, FOX, F, TGC, RD</td>
<td>Smoked fish</td>
</tr>
<tr>
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<td>mec(A)</td>
<td>NC</td>
<td>DA, FOX, F, TGC, SXT, C, RD, CN, LZD</td>
<td>Sausage</td>
</tr>
<tr>
<td>S. xylosus 629G</td>
<td>mec(A)</td>
<td>NC</td>
<td>E, DA, FOX, F, W, TGC, RD, CN, LZD</td>
<td>Cheese</td>
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Staphylococcus spp. 777bG tet(K) TE Sausage
The strain resistance to antibiotics of the macrolide-lincosamide-streptogramin (MLS) group is also significant. Although those antibiotics are not used in animal treatment, frequent administration of macrolide-class tylosin to animals resulted in the development of cross-resistance to the MLS group (30). Among the examined isolates, strains resistant to antibiotics of the MLS group made up a significant number, of which 46 strains revealed resistance to DA, 10 strains to E, and 26 strains to QDA (Table 1). Because QDA can be used to treat MRSA infection in humans (11), our study suggests a need to monitor the QDA resistance in staphylococci of retail food and to understand the potential of QDA-resistant strains to cause human disease.

Many of our isolates carried two TE resistance determinants, which reveals a distribution of this type of resistance. The carriage of multiple tet genes was commonly found in individual gram-positive bacteria (15, 23). TE resistance can be conferred by genes encoding efflux proteins tet(K) and tet(L) or ribosomal protection proteins tet(M), tet(O), and tet(S). In addition, it can be conferred by an unknown mechanism encoded by tet(U) (4, 15). The high incidence of tet(M) and tet(L) genes in the isolated staphylococci can be explained by their usual genetic locations. In fact, the presence of the tet(L) gene on small multicopy plasmids and tet(M) on conjugative transposons (Tn916-Tn1545 family) contributes to the spread of these determinants (4). The Tn916 transposon, which is usually associated with tet(M), was detected in only 13 of 38 isolates (Table 2). Our results are not in agreement with those of Bhargava and Zhang (3), who detected tet(M) in 36 of 56 investigated strains, among which 31 also had Tn916.

Many studies have shown a comparably low correlation of phenotypic and genotypic detection of antibiotic resistance. Resch et al. (22) found staphylococci that were phenotypically resistant to TE, but tet(L) and tet(M) could not be detected. Our results are partially in agreement with this finding because tet genes were not detected in three phenotypically resistant strains, whereas the erm(B) gene was not found in any of the E-resistant strains (Table 2). Discrepancies between phenotype resistance and the presence of genes conferring resistance may be due to the presence of a so-called silent gene that is expressed only in vivo or may be due to the fact that detection by PCR of a single gene inside an operon may overlook the absence of other genes that are necessary for phenotypic expression. The reverse situation may be due to the presence of other genes conferring resistance to these antibiotics, erm(A), erm(C), tet(O), and tet(S), which were not examined. Sekiguchi et al. (26) also found discordance among TE and/or E genes and phenotypic sensitivity. The authors stated that this discordance might be brought about by mutations in the coding or promoter region of the PCR-detected genes.

Our finding that none of the 10 E-resistant isolates had any resistance genes identified was not surprising, given previous reports (28), although it could also be due to the limited number of genes tested. The predominant E resistance genes in Staphylococcus isolated from various sources commonly found in E-resistant staphylococci were erm(A) and erm(C) (9, 16, 28), erm(B) is less common in Staphylococcus than in Enterococcus and Streptococcus (3).

The results of our study indicated that retail RTE food could be considered one of the main routes for transmission of antibiotic-resistant bacteria harboring multiple antibiotic resistance genes to human populations. Genes encoding antibiotic resistance are usually located on mobile genetic elements; this means that their transfer to human pathogens is possible. Considering this, screening for antibiotic resistance in bacteria from RTE food, which does not need thermal processing before consumption, is important.

ACKNOWLEDGMENT

This research was supported by a grant from the Ministry of Science and Higher Education, Poland, no. N N312 236138.

REFERENCES