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Original article

Prevalence of antibiotic resistance genes in staphylococci isolated from ready-to-eat meat products

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Abstract

Prevalence of *mecA, bla*Z, *tet*O/K/M, *erm*A/B/C, *aph*, and *van*A/B/C/D genes conferring resistance to oxacillin, penicillin, tetracycline, erythromycin, gentamicin, and vancomycin was investigated in 65 staphylococcal isolates belonging to twelve species obtained from ready-to-eat porcine, bovine, and chicken products. All coagulase negative staphylococci (CNS) and *S. aureus* isolates harbored at least one antibiotic resistance gene. None of the *S. aureus* possessed more than three genes, while 25% of the CNS isolates harbored at least four genes encoding resistance to clinically used antibiotics. In 15 CNS isolates the *mec*A gene was detected, while all *S. aureus* isolates were *mec*A-negative. We demonstrate that in ready-to-eat food the frequency of CNS harboring multiple antibiotic resistance genes is higher than that of multiple resistant *S. aureus*, meaning that food can be considered a reservoir of bacteria containing genes potentially contributing to the evolution of antibiotic resistance in staphylococci.

Key words: coagulase-negative staphylococci, *S. aureus,* RTE food, antibiotic resistance

Introduction

Staphylococci are human commensals and a frequent cause of infections including life-threatening device-associated bacteriemias (Kloos et al. 1994, Lowy 1998). More than 50 *Staphylococcus* species and subspecies have been characterized to date (www.dsmz.de/dsmz). The genus *Staphylococcus* is divided into coagulase-positive and coagulase-negative (CNS) species according to their ability to coagulate plasma. The CNS group includes species considered as positive food flora, thus being widely applied in industry e.g. as the component of meat starter cultures (Irlinger 2008). However, recent research demonstrates that food can be a reservoir of antibiotic resistant CNS and *S. aureus* strains (Martin et al. 2006, Simeoni et al. 2008). Over the past years, due to the extensive use of antimicrobials in public health and animal husbandry, the antibiotic resistance of coagulase-positive staphylococci, especially of *S. aureus* and some CNS species, such as *S. epidermidis*, has dramatically increased. Infections with methicilin-resistant *S. aureus* (MRSA) and methicilin-resistant *S. epidermidis* (MRSE) constitute a serious public health problem around the world. Genes encoding antibiotic resistance are usually located on mobile genetic

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elements, allowing their horizontal transfer to pathogenic staphylococci (Resch et al. 2008). The risk of transfer becomes real, as some species, such as *S. xylosus, S. carnosus* and *S. pasteuri*, being components of starter cultures, are used at high concentrations during production of fermented food (Hugas and Monfort 1997).

The aim of this study was to determine the prevalence of antibiotic resistance genes among CNS and *S. aureus* isolates from ready-to-eat meat products.

Materials and Methods

Isolation and identification of staphylococci

Seventy samples of ready-to-eat porcine, bovine and chicken meat products were screened for presence of staphylococci. The samples were taken during a four-months period from five randomly selected supermarkets in Wrocław. Staphylococci from food samples were cultured on Giolitti-Cantoni enrichment broth and then subcultured on Baird-Parker agar. One CNS and/or one *S. aureus* isolate per sample was used for further characterization. The *S. aureus* and CNS isolates were identified on the basis of catalase, clumping factor and coagulase tube tests. The CNS strains were identified to species level by API STAPH (bioMerieux, Warsaw, Poland), and partial 16S rDNA sequence analysis using primers from htpp://rdna4.ridom.de/static/primer.html. *S. aureus* isolates were screened with PCR using *S. aureus*-specific primers for the *nuc* gene, encoding thermonuclease (Martin et al. 2003).

Preparation of bacterial DNA

Two millilitres of bacterial cell suspension from an overnight culture grown in brain-heart infusion (BHI) broth were centrifuged for 5 min at 12,000 × g and suspended in 100 µl of 100 mM Tris-HCl buffer, pH 7.4, containing 10 µg of lysostaphin (A&A Biotechnology, Gdańsk, Poland). After 30-minute incubation at 37°C, 10 µl of 10% SDS was added and the sample was incubated for another 30 min at 37°C. Two hundred µL of 5 M guanidine hydrochloride were added and the sample was mixed and incubated at room temperature for 10 min. The DNA was extracted using phenol and chloroform, precipitated with ethanol, and dissolved in water.

Detection of antimicrobial resistance genes

All CNS and *S. aureus* strains were tested for the presence of *mec*A (oxacillin resistance), *bla*Z (penicillin resistance), *tet*O/K/M (tetracycline resistance), *erm*A/B/C (erythromycin resistance), *aph* (gentamicin resistance) and *van*A/B/C/D (vancomycin resistance) genes using the primers and conditions described by Milheiriço (2007) and Rizotti (2005). Reference MRSA and MSSA strains were kindly provided by Professor Waleria Hryniewicz from the National Medicines Institute, Warsaw, Poland. To verify the results for other antibiotic resistance genes, one amplicon from each gene specific PCR was sequenced.

Results

Identification of staphylococcal species

Sixty-five staphylococcal isolates belonging to twelve species were obtained from 70 ready-to-eat (RTE) food products. *S. aureus* (n = 25) and *S. epidermidis* (n = 13) constituted dominant species in the examined foodstuffs. The remaining species were *S. pasteuri* (n = 7), *S. haemolyticus* (n = 4), *S. carnosus* (n = 2), *S. saprophyticus* (n = 5), *S. sciuri* (n = 3), *S. chromogenes* (n = 2), *S. capitis* (n = 3), *S. xylosus* (n = 1), *S. equorum* (n = 1) and *S. lugdunensis* (n = 1).

Content of antibiotic resistance genes

All analyzed staphylococci were *van*A/B/C/D negative. Fifteen (37%) CNS isolates were *mec*A positive. None of the twenty-five *S. aureus* isolates possessed the *mec*A gene. Thirty seven (92%) CNS isolates and twenty-four (96%) *S. aureus* isolates possessed the *bla*Z gene. Eleven (44%) *S. aureus* isolates harbored the *tet*O/K/M genes. Among the CNS population twenty-four (60%) isolates harbored the *tet*O/K/M genes. The *erm*A/B/C genes were detected in 15 (60%) *S. aureus* and 17 (42%) CNS isolates. None of the *S. aureus* isolates possessed the *aph* gene, while among the CNS group nine (22%) isolates were *aph*-positive (Table 1).

All CNS and *S. aureus* isolates possessed at least one of the antibiotic resistance genes. In the *S. aureus* population 56% and 24% of isolates harbored two and three antibiotic resistance genes, respectively. None of the *S. aureus* isolates possessed more than three resistance genes. In the CNS group 25% of isolates harbored at least four genes encoding resistance to clinically used antibiotics. Two *S. epidermidis*, one S. *haemolyticus* and one *S. chromogenes* isolates possessed five antibiotic resistance genes (Table 2).

Table 1. Number of isolates harboring antibiotic resistance genes.

Species	Number of isolates	Number of isolates harboring antibiotic resistance genes					
		blaZ	*Mec*A	*van* A/B/C/D	*tet* O/K/M	*erm* A/B/C	*aph*
S. epidermidis	13	13 (100%)	6 (46%)	0	6 (46%)	5 (38%)	2 (15%)
S. pasteuri	7	7 (100%)	1 (14%)	0	5 (71%)	3 (43%)	1 (14%)
S. saprophyticus	4	2 (50%)	0	0	2 (50%)	3 (75%)	0
S. haemolyticus	4	4 (100%)	3 (75%)	0	4 (100%)	1 (25%)	2 (50%)
S. sciuri	3	3 (100%)	3 (100%)	0	1 (33%)	0	1 (33%)
S. capitis	3	2 (67%)	0	0	1 (33%)	2 (67%)	1 (33%)
S. chromogenes	2	2 (100%)	1 (50%)	0	2 (100%)	1 (50%)	1 (50%)
S. carnosus	1	1 (100%)	0	0	0	0	0
S. xylosus	1	1 (100%)	0	0	1 (100%)	1 (100%)	1 (100%)
S. equorum	1	1 (100%)	0	0	1 (100%)	0	0
S. lugdunensis	1	1 (100%)	1 (100%)	0	1 (100%)	1 (100%)	0
Total CNS	40	37 (92%)	15 (37%)	0	24 (60%)	17 (42%)	9 (22%)
S. aureus	25	24 (96%)	0	0	11 (44%)	15 (60%)	0

Table 2. Number of resistance genes carried.

Species	Number of isolates	Number of resistance genes carried				
		1 gene	2 genes	3 genes	4 genes	5 genes
S. aureus	25	5	14	6	–	–
S. epidermidis	13	2	7	2	–	2
S. pasteuri	7	1	3	2	1	–
S. saprophyticus	4	2	1	1	–	–
S. haemolyticus	4	–	1	1	1	1
S. sciuri	3	–	2	–	1	–
S. capitis	3	2	–	–	1	–
S. chromogenes	2	–	1	–	–	1
S. carnosus	1	1	–	–	–	–
S. xylosus	1	–	–	–	1	–
S. equorum	1	–	1	–	–	–
S. lugdunensis	1	–	–	–	1	–
Total CNS	40	8	16	6	6	4

Discussion

In recent decades, CNS have been among the most frequently isolated bacteria in clinical microbiology laboratories (Pfaller and Herwaldt 1988, Patrick 1990). Many CNS species are responsible for serious infections, especially in immunocompromised patients and premature children (Karchmer et al. 1983, Goldmann et al. 1993). *S. epidermidis, S. pasteuri, S. saprophyticus, S. haemolyticus* and *S. capitis* being dominant species identified in this study are common opportunistic human pathogens (Götz et al. 2006, Irlinger 2008). Several CNS isolates are found to be resistant to multiple antimicrobials (Kloos et al. 1994, Kozitskaya et al. 2004). Occurrence of *S. epidermidis* isolates with decreased sensitivity to drugs of "last resort", i.e. glycopeptide antibiotics, has already been reported (Watanakunakorn 1985, Walsh et al. 2001).

The main source of antibiotic-resistant staphylococci are humans, and person-to-person transmission is considered the main route of contamination. They can be transmitted to foodstuffs if insufficient care is taken during food production (Baird-Parker 1990, Marples et al. 1990, Noble 1990). Antibiotic resistance of food-derived *S. aureus* has been extensively investigated, but prevalence of antibiotic resistance determinants in CNS from food remains largely unrecognized. All CNS and *S. aureus* isolates tested in this study possessed at least one antibiotic resistance gene. More than 90% of staphylococci possessed the *blaZ* gene encoding resistance to penicillin. Resistance to penicillin is explained by the hyperproduction of β-lactamase and is frequent among staphylococci of clinical origin (Hryniewicz et al. 2010). Genes encoding resistance to tetracycline were harbored by 60% of CNS and 44% of *S. aureus* isolates. This kind of resistance is also common among staphylococci isolated

from hospital environments (Koksal et al. 2009, Hryniewicz et al. 2010). None of the *S. aureus* isolates studied here possessed the *mec*A gene. Resistance to oxacillin conferred by the *mec*A gene is considered an important pathogenic trait of hospital and community-acquired staphylococci. In this study 15 (37%) CNS isolates were *mec*A-positive. Resch et al. (2008) showed that 22% of food-associated CNS were oxacillin-resistant and most of these strains were found among *S. xylosus*, *S. succinus* and *S. equorum*. Similar results were obtained for CNS isolated from Spanish fermented sausages and bovine milk in which *mec*A-positive *S. epidermidis* accounted for 28% and 32%, respectively (Martin et al. 2006, Sawant et al. 2009). All *S. haemolyticus* and *S. sciuri* isolates in this study, and 46% of the *S. epidermidis* strains possessed the *mec*A gene. Twenty five percent of CNS harbored at least four antibiotic resistance genes. In contrast, none of the *S. aureus* possessed more than 3 genes encoding resistance to clinically used antibiotics.

Food is generally considered a minor reservoir of multiresistant *S. aureus* strains if compared with its carriage rates in human or animals (Wertheim et al. 2005, Leonard et al. 2008, Weese 2010). We nonetheless demonstrate that ready-to-eat meat products can be a considerable reservoir of CNS harboring multiple resistance genes. Genes encoding antibiotic resistance are usually located on mobile genetic elements, which means that their transfer to pathogenic staphylococcal species is possible. According to Kloos (1997) and Szewczyk et al. (2004) *S. hominis,* and *S. cohnii* frequently present in clinical samples are considered as a reservoir of resistance genes in the environment. It is surmised that non-*S. aureus* staphylococci carrying antibiotic resistance genes significantly contribute to the evolution of MRSA in both hospital and community settings (Kassem 2011).

We demonstrate that in ready-to-eat food the frequency of CNS harboring multiple antibiotic resistance genes is higher than that of multiple resistant *S. aureus*, meaning that food can be considered a reservoir of bacteria carrying genes potentially contributing to the evolution of antibiotic resistance in pathogenic staphylococcal species.

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