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Coagulase-negative staphylococci (CoNS) isolated from ready-to-eat food of animal origin – Phenotypic and genotypic antibiotic resistance

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ABSTRACT

The aim of this work was to study the pheno- and genotypical antimicrobial resistance profile of coagulase negative staphylococci (CoNS) isolated from 146 ready-to-eat food of animal origin (cheeses, cured meats, sausages, smoked fishes). 58 strains were isolated, they were classified as *Staphylococcus xylosus* ($n = 29$), *Staphylococcus epidermidis* ($n = 16$); *Staphylococcus lentus* ($n = 7$); *Staphylococcus saprophyticus* ($n = 4$); *Staphylococcus hyicus* ($n = 1$) and *Staphylococcus simulans* ($n = 1$) by phenotypic and genotypic methods. Isolates were tested for resistance to erythromycin, clindamycin, gentamicin, cefoxitin, norfloxacin, ciprofloxacin, tetracycline, tigecycline, rifampicin, nitrofurantoin, linezolid, trimetoprim, sulphamethoxazole/trimethoprim, chloramphenicol, quinupristin/dalfopristin by the disk diffusion method. PCR was used for the detection of antibiotic resistance genes encoding: methicillin resistance – *mec*A; macrolide resistance – *erm*(A), *erm*(B), *erm*(C), *mrs*(A/B); efflux proteins *tet*(K) and *tet*(L) and ribosomal protection proteins *tet*(M). For all the *tet*(M)-positive isolates the presence of conjugative transposons of the Tn*916*–Tn*1545* family was determined. Most of the isolates were resistant to cefoxitin (41.3%) followed by clindamycin (36.2%), tigecycline (24.1%), rifampicin (17.2%) and erythromycin (13.8%). 32.2% staphylococcal isolates were multidrug resistant (MDR). All methicillin resistant staphylococci harboured *mec*A gene. Isolates, phenotypic resistant to tetracycline, harboured at least one tetracycline resistance determinant on which *tet*(M) was most frequent. All of the isolates positive for *tet*(M) genes were positive for the Tn*916*–Tn*1545* -like integrase family gene. In the erythromycin-resistant isolates, the macrolide resistance genes *erm*(C) or *msr*(A/B) were present. Although coagulase-negative staphylococci are not classical food poisoning bacteria, its presence in food could be of public health significance due to the possible spread of antibiotic resistance.

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1. Introduction

Most research concerning antibiotic resistance of staphylococci isolated from food of focuses on the *Staphylococcus aureus* species, whereas less attention is paid to the group of coagulase-negative staphylococci (CoNS) (Gao et al., 2012). Due to the fact that for many years CoNS were considered non-pathogenic, in routine laboratory tests, CoNS are very often identified only at the genus level, while coagulase-positive strains are selected for further analyses. The results of recent research also suggest that CoNS can be potentially pathogenic (Gillespie et al., 2009). Researchers are increasingly often identifying *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus saprophyticus* as the cause of nosocomial infections in humans (Mazzariol et al., 2012; Piette and Verschraegen, 2009) and to *Staphylococcus chromogenes*, *Staphylococcus simulans* and *Staphylococcus xylosus* as a cause of infections in animals (Taponen et al., 2006; Unal and Cinar, 2012). Additionally, it is also claimed that coagulase-negative staphylococci are a significant reservoir of resistance genes (Corrente et al., 2009; Podkowik et al., 2012). To some extent, the role of staphylococci in food is of a dual nature. Among CoNS, species of *Staphylococcus carnosus*, *S. xylosus*, *S. condimenti*, *Staphylococcus equorum*, *Staphylococcus piscifermentans*, *Staphylococcus succinus* play a significant role in food production. *S. carnosus* and *S. xylosus* strains are used as starter cultures for the production of fermented meat products such as fermented sausages and play a significant

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role in defining the colour and in developing organoleptic features according to their proteolytic and lipolytic abilities.

Pursuant to the current Commission Regulation (EC) of 15 November 2005 as amended on microbiological criteria for foodstuffs (EC Regulation No. 2073/2005), the hygiene criteria include only the examination of the number of coagulase-positive staphylococci. The acceptable limit of these bacteria in food has been established as 10^5 cfu/g. The Regulation does not refer to the occurrence of other *Staphylococcus* species in food. Food is often a reservoir of these bacteria, and their common occurrence results primarily from resistance to unfavourable environmental conditions during the production processes, food storage and high adaptation abilities of those micro-organisms (Chajęcka-Wierzchowska et al., 2014).

The spread of resistance to antimicrobial agents in staphylococci is largely due to the acquisition of plasmids and/or transposons (Lozanoa et al., 2012). In staphylococci, the conjugative transfer of resistance determinants is usually mediated by conjugative plasmids which spread resistance determinants between species and genera (Malachowa and DeLeo, 2010). Besides transferring the resistance determinants, they can mobilize nonconjugative plasmids, recombine with nonconjugative plasmids to form new plasmids, or acquire and transfer resistance transposons (Khan et al., 2000).

The resistance mechanism in methicillin resistance CoNS is related to the presence of *mec*A gene. The *mec*A gene is located on a mobile genetic element called Staphylococcal Cassette Chromosome *mec* (SCC*mec*). Horizontal, interspecies transfer of this element could be an important factor in the dissemination of methicillin-resistant *S. aureus* (MRSA) (Bloemendaal et al., 2010). Tetracycline resistance coded by a wide variety of determinants in different staphylococcal species is also frequently encountered (De Vries et al., 2009; Lim et al., 2012). The spread of antibiotic resistance among CoNS (harbouring resistance genes) from ready to eat food may represent a hazard for human health through transfer of resistance genes between staphylococcal species, and through direct transmission of resistant pathogens to humans (Walther and Perreten, 2007).

The aim of this study was to determine phenotypic antimicrobial resistance profiles of CoNS isolated from food of animal origin, with emphasis on antibiotics of clinical relevance, i.e. β-lactams, cefoxitin and macrolide–lincosamide–streptogramins (MLS) compounds; to assess associations between species and resistance profiles furthermore detection of resistance gene *tet*(M), *tet*(K), *tet*(L), *erm*(A), *erm*(B), *erm*(C), *mrs*(A/B) and *mec*A.

2. Materials and methods

2.1. Bacterial strains

Staphylococci were isolated from 146 samples obtained from various retail ready-to-eat products from animal origin (cheeses, cured meats, sausages, smoked fishes) obtained from several local markets in Olsztyn, Poland. Food samples (10 g) were homogenized in 90 ml buffered peptone water (Merck, Germany), incubated overnight at 37 °C and streaked on selective plates containing Mannitol Salt Phenol-red Agar (Merck, Germany). Mannitol (+) colonies were differentiated into coagulase-positive and coagulase-negative with a test detecting the production of a clumping factor (Staphylase Test Kit, Oxoid, United Kingdom) and production of coagulase on the RPF medium (Biomerieux, France) with rabbit blood plasma and fibrinogen. After the initial phenotypic analysis, coagulase-negative strains were identified at the genus level by the Multiplex PCR method according to the protocols of Morot-Bizot et al. (2004). DNA of the strains was isolated from single colonies. The isolation kit applied was based on selective binding and elution of nucleic acid on a mini column – Genomic Mini (A&A Biotechnology, Poland), with previous stage of cell wall digestion (Lysostaphin 10 mg/ml) (A&A Biotechnology, Poland). Amplification was carried out in a MJ Mini thermal cycler (BIO-RAD, Poland). The research used starters synthesised in the Laboratory of DNA Sequencing and Oligonucleotide Synthesis (Institute of Biochemistry and Biophysics of the Polish Academy of Sciences in Warsaw, Poland) – Table 1. Electrophoretic separation: 100 V – 5 min; 70 V – 60 min (Cleaver Scientific, UK) was carried out on 1.5% agarose gel (Promega, Poland) with the addition of ethidium bromide 0.5 μg/10 ml (MP Biomedicals, Poland). Positive controls included reference strains: *S. aureus* ATCC 43300, *S. xylosus* ATCC 29971, *S. saprophyticus* ATCC 49453 and *S. epidermidis* ATCC 49461. Strains identified by the PCR method only at the genus level were identified using API STAPH (Biomerieux, France).

2.2. Phenotypic antibiotic resistance

Resistance to antibiotics was examined according to the guidelines of the National Reference Centre for Antimicrobial Susceptibility and internationally recognized standards of the Clinical and Laboratory Standards Institute (CLSI, 2010). Determinations were carried out using the diffusion disk method on Müeller-Hinton agar (Merck, Germany). The following discs (Oxoid, Poland) were used: erythromycin (E-15 μg), clindamycin (DA-2 μg), gentamicin (CN-120 μg), cefoxitin (FOX-30 μg), norfloxacin (NOR-10 μg), ciprofloxacin (CIP-5 μg), tetracycline (TE-30 μg), rifampicin (RD-5 μg), nitrofurantoin (F-300 μg), linezolid (LZD-30 μg), chloramphenicol (C-30 μg), trimetoprim (W-5 μg), trimetoprim/sulfamethoxazole (SXT-25 μg), quinupristin/dalfopristin (QDA-15 μg), tigecycline (TGC-1 μg). *Escherichia coli* ATCC 25922 and *S. aureus* ATCC 29213 were used as reference strains for antibiotic disc control.

Table 1
Oligonucleotides used in PCR reactions.

Species/gene	Primer sequence (5'→ 3')	Amplicon size (bp)	Source
Staphylococcus spp.	TIACCATTTCAGTACCTTCTGGTAA GGCCGTGTTGAACGTGGTCAAATCA	370	Morot-Bizot et al., 2004
S. aureus	CGTAATGAGATTTCAGTAG ATAATACAACA AATCTTTGTCGGTACACGA TATTCTTCACG	107	
S. xylosus	AACGCGCAACAGCAATTACG AACGCGCAACGTGATAAAATTAATG	539	
S. epidermidis	CAAAAGAGCGTGGAGAAAAGTATCA ATCAAAAAGTTGGCGAACCTTTTCA	124	
S. saprophyticus	ACGGGCGTCCACAAAATCAATAGGA TCAAAAAGTTTTCTAAAAAATTTAC	220	
*mec*A	AAAATCGATGGTAAAGGTTGGC AGTTCTGGCACTACCGGATTTGC	533	Barski et al., 1996
tet(L)	TGGTGGAATGATAGCCCATT CAGGAATGACAGCACGCTAA	229	Rizzotti et al., 2005
tet(M)	GTGGACAAAGGTACAACGAG CGGTAAAGTTCGTCACACAC	406	
erm(B)	TGGTATTCCAAATGCGTAATG CTGTGGTATGGCGGGTAAGT	745	
tet(K)	TTATGGTGGTTGTAGCTAGAAA AAAGGGTTAGAAACTCTTGAAA	348	Gevers et al., 2003
int (Tn*916*/Tn*1545*)	GCGTGATTGTATCTCACT GACGCTCCTGTTGCTTCT	1028	Doherty et al., 2000
erm(A)	TCTAAAAAGCATGTAAAAGAA TGATTATTATTTGATAGCTTC	645	Sutcliffe et al., 1996
erm(C)	TCAAAACATAATATAGATAAA TAACTGCTAAATTTGTTATAATCG	642	
mrs(A/B)	GCAAATGGTGTAGGTAAGACAACT TAAAACAAATGTAGTGTACTA	399	

2.3. Analysis of the molecular mechanisms of antibiotic resistance

For all isolates, the presence of the genes encoding efflux proteins (*tet*(K) and *tet*(L)) and genes encoding ribosomal protection proteins (*tet*(M)) was performed. The tetracycline efflux gene *tet*(K) amplification was performed according Gevers et al. (2003). PCR detection of resistance to tetracyclines – *tet*(M), *tet*(L) and macrolides – *erm*(B) was determined by multiplex PCR (triplex) using the specific primers and the conditions reported by Rizzotti et al. (2005). For all the *tet*(M)-positive isolates the presence of conjugative transposons of the Tn*916*–Tn*1545* family was determined by using primers targeting the integrase gene *int* according to Doherty et al. (2000). Detection of *mec*A gene were carried out according to protocols described by Barski et al. (1996). Detection of *erm*(A), *erm*(C) and *mrs*(A/B) gene were carried out according to Sutcliffe et al. (1996). In all PCRs determining antimicrobial resistance genes, strains from Department of Industrial and Food Microbiology and American Type Culture Collection were used as positive controls: *Enterococcus faecalis* 20138 EK (positive for *tet*(M), *tet*(L), *erm*(B)); *E. faecalis* 15555 EK (positive for *tet*(K)) and *S. epidermidis* ATCC 49461 (*mec*A–positive). The amplicons were evaluated by 1.5% agarose gel electrophoresis followed by staining in ethidium bromide (0.5 mg/mL), visualized on UV transilluminator.

3. Results

3.1. Prevalence of CoNS in ready-to-eat food

In the present study fifty-eight strains of coagulase-negative staphylococci (CoNS) isolated from ready-to-eat food of animal origin bought in the retail were tested. The following species were identified: *S. xylosus* ($n = 29/50\%$), *S. epidermidis* ($n = 16/27.6\%$), *Staphylococcus lentus* ($n = 7/12.1\%$), *S. saprophyticus* ($n = 4/6.9\%$), *Staphylococcus hyicus* ($n = 1/1.7\%$) and *S. simulans* ($n = 1/1.7\%$).

3.2. Phenotypic and genotypic antibiotic resistance

Resistance to at least one antibiotic was observed in 33 strains (56.9%), of which 13 strains were isolated from cured meat, 12 from cheeses and 8 from smoked fish (Table 2). Most isolates revealed a resistance to cefoxitin – FOX ($n = 24/41.3\%$) of which all harbour *mec*A gene (Table 2). 83.3% methicillin resistant strains ($n = 24$) showed at the same time resistance to clindamycin (DA), 56% to tetracycline (TE + TGC) and 40% to rifampicin (RD). A significant percentage of isolates were resistant to antibiotics of the MLS group ($n = 33/56.9\%$), of which 21 strains (36.2%) revealed resistance to clindamycin (DA), 8 strains (13.8%) to erythromycin (E) and 4 strains (6.9%) to quinupristin/dalfopristin (QDA). Out of 8 staphylococci strains resistant to erythromycin, 7 were positive for the *msr*(A/B) gene and 1 for *erm*(C) gene. Twenty strains (34.5%) were resistant to antibiotics of the tetracycline group, of which 14 strains (24.1%) were resistant to tigecycline (TGC) and 6 strains (10.3%) to tetracycline (TE). Two of the examined strains were resistant at the same time to both antibiotics of the tetracycline group. All of the isolates phenotypic resistant to tetracycline harboured at least one tetracycline resistance determinant on which *tet*(M) was most frequent. The *tet*(L) genes was also appear but were less common. *Tet*(K) gene was always associated with *tet*(M) and *tet*(L). All of the isolates positive for *tet*(M) genes were positive for the Tn*916*/Tn*1545*-like integrase family gene. Ten strains (17.2%) were resistant to rifampicin (RD). Resistance to other antibiotics under examination was observed in groups ranging from 2 (3.4%) to 7 (12.1%) of the examined strains (Table 2). Out of the 58 strains under analysis, 19 strains (32.8%) revealed multi-drug resistance (MDR) defined as resistance to antibiotics belonging to three or more classes. Two out of the examined strains (belonging to the *S. xylosus* species) revealed simultaneous resistance to antibiotics of nine various classes. Most MDR strains (31.6%) revealed simultaneous resistance to four classes of antibiotics. All MDR strains (100%) were resistant to cefoxitin (FOX) and the majority (84.2%) were resistant to clindamycin (DA) and tigecycline (TGC) (52.6%). The most frequently-occurring phenotype in MDR strains was DA-FOX-TGC (Table 3).

Table 2
Antimicrobial resistance identified in CoNS.

Species	Phenotype	Antibiotic resistance genes	Source
S. epidermidis	DA, FOX, TGC, RD, CN, QD	*mec*A	cheese
S. epidermidis	FOX, W, TGC, SXT, RD	*mec*A	cheese
S. epidermidis	E, FOX, W	*mrs*(A/B), *mec*A	cheese
S. epidermidis	DA, FOX, LZD, QD	*mec*A	fish
S. epidermidis	DA, FOX, TGC, TE,QD	*tet*(L), *mec*A	fish
S. epidermidis	E, DA	*mrs*(A/B)	fish
S. epidermidis	FOX, F, W, TGC, SXT	*mrs*(A/B), *mec*A	fish
S. epidermidis	DA, TGC, CIP	–	cured meat
S. epidermidis	E, DA, FOX, NOR, W, TGC, RD	*mec*A	cured meat
S. epidermidis	FOX	*tet*(L), *tet*(M), *tet*(K), *mec*A, *int*	cured meat
S. epidermidis	C	*mec*A	cured meat
S. epidermidis	DA, FOX, LZD, QD	*tet*(L), *tet*(M), *mec*A, *int*	cured meat
S. hyicus	TE	*tet*(M), *int*	cheese
S. lentus	DA, FOX, F, CN	*mec*A	cheese
S. lentus	DA, FOX, F, RD, CN	*mec*A	cheese
S. lentus	DA, FOX	*mec*A	cured meat
S. saprophyticus	E, DA, FOX, TE, CIP, CN	*tet*(L), *tet*(M), *tet*(K), *mec*A, *int*	cheese
S. simulans	E, TE	*tet*(M), *erm*(C), *int*	fish
S. xylosus	DA, FOX	*mec*A	cheese
S. xylosus	E	*mrs*(A/B)	cheese
S. xylosus	E, DA, FOX, F, W, TGC, RD, CN, LZD	*mrs*(A/B), *mec*A	cheese
S. xylosus	FOX	*mec*A	cheese
S. xylosus	DA, FOX	*mec*A	cheese
S. xylosus	DA, FOX, TGC, CIP, RD	*mec*A	fish
S. xylosus	DA, FOX, TGC, RD, CN, LZD	*mec*A	fish
S. xylosus	E, DA, FOX, F, TGC, RD	*mrs*(A/B), *mec*A	fish
S. xylosus	DA, FOX, F, TGC, SXT, C, RD, CN, LZD	*tet*(L), *mec*A	cured meat
S. xylosus	DA, FOX, NOR	*mec*A	cured meat
S. xylosus	DA, FOX, TGC, RD, LZD	*tet*(L), *mec*A	cured meat
S. xylosus	DA, FOX, TGC, TE,	*tet*(L), *tet*(M), *tet*(K), *mec*A	cured meat
S. xylosus	TE	*tet*(M), *int*	cured meat
S. xylosus	W, SXT, C	*mrs*(A/B)	cured meat
S. xylosus	TGC	–	cured meat

Abbrevations: E – erythromycin, DA – clindamycin, CN – gentamicin, FOX – cefoxitin, NOR – norfloxacin, CIP – ciprofloxacin, TE – tetracycline, TGC – tigecycline, RD – rifampicin, F – nitrofurantoin, LZD – linezolid, W – trimetoprim, SXT – trimetoprim/sulfamethoxazole, C – chloramphenicol, QDA – quinupristin/dalfopristin; black – resistance phenotypes; grey – susceptible phenotypes.

4. Discussion

Little information is available regarding the diversity of antibiotic resistance and resistance genes in coagulase-negative *Staphylococcus* species isolated from ready-to-eat. This study provides the analysis of antibiotic resistance including genotypes of a variety of staphylococci isolated from retail ready-to-eat food from animal origin. The identified Staphylococcal species (*S. xylosus*, *S. epidermidis*, *S. lentus*, *S. saprophyticus*, *S. hyicus* and *S. simulans*) are commonly in food and they are also associated with farm animals. However, some of the authors indicate that occurrence of

Table 3
Distribution of antibiotic resistance patterns among MDR-CoNS.[a]

DA, FOX, F, TGC, SXT, C, RD, CN, LZD	9 class ($n = 2$)
E, DA, FOX, F, W, TGC, RD, CN, LZD	
E, DA, FOX, NOR, W, TGC, RD	7 class ($n = 1$)
DA, FOX, TGC, RD, CN, LZD	6 class ($n = 4$)
DA, FOX, TGC, RD, CN, QD	
E, DA, FOX, F, TGC, RD	
E, DA, FOX, TE, CIP, CN	
DA, FOX, F, RD, CN	5 class ($n = 4$)
DA, FOX, TGC, CIP, RD	
DA, FOX, TGC, RD, LZD	
DA, FOX, TGC, TE,QD	
FOX, F, W/SXT, TGC	4 class ($n = 5$)
FOX, W/SXT, TGC, RD	
DA, FOX, F, CN	
DA, FOX, LZD, QD	
DA, FOX, TGC, TE,	
DA, FOX, NOR	3 class ($n = 3$)
E, FOX, W	
DA, TGC, CIP	

[a] MDR (ang. multidrug-resistance) coagulase negative staphylococci resistance to antibiotics belonging to three or more classes; n-number of isolates.

staphylococci in ready-to-eat food might be related to human contamination rather that contamination with animal origin (Fontes et al., 2013). Additionally, Schlegelova et al. (2008) claims that this species is more often found in dairy and meat products than in raw materials from which they are produced. This can suggest re-infection during the production process, due to the common occurrence of *S. epidermidis* on employees' hands and its ability to produce a biofilm and to survive in production spaces.

In recent years, systematic growth in the number of antibiotic resistant strains in the human environment has been observed. The researchers suggest that food could be appropriate environment for resistant and multi-resistant strains and the food chain can play a key role in the transmission of resistance between the environment and humans (Chajęcka-Wierzchowska et al., 2012). In the tests performed, resistance to at least one antibiotic was observed in more than half of isolates (Table 2). Most stains revealed a resistance to cefoxitin which in case of strains isolated from food is an alarming phenomenon since it determines strains of the MR-CoNS (methicillin resistant coagulase-negative *Staphylococcus*) phenotype. Methicillin resistant strains 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). This is confirmed, among others, by examinations of the resistance profiles of coagulase-negative staphylococci carried out by Bhargava and Zhang (2012) – in which all methicillin resistant strains revealed phenotypical resistance to penicillin and oxacillin at the same time. Our analysis of the results revealed another interesting relation, namely that even 83.3% methicillin resistant strains showed at the same time resistance to clindamycin, tetracycline and rifampicin.

Currently, from the clinical point of view, methicillin-resistant staphylococci constitute a very serious therapeutic problem (Huber et al., 2011). The resistance mechanism in MR-CoNS is related to the presence of *mec*A gene situated within the SCCmec cassette (staphylococcocal cassette chromosome *mec*) located on the bacteria chromosome. The *mec*A gene encodes PBP2a (PBP22'), a penicillin-binding protein, which results in phenotypic resistance. The results of our research have revealed the presence of the *mec*A gene which encodes resistance to methicillin in all strains revealing phenotypical resistance to cefoxitin. The *mec*A gene was carried by 26 of the 33 isolates resistant to at least one antibiotic. One strain of *S. epidermidis* which was found to carry *mec*A gene was phenotypically cefoxitin-susceptible (Table 2). This situation is in accordance with other authors (Vyletělová et al., 2011; Souza Antunes et al., 2007). Only 84% of *mec* A-positive strains isolated in the study of Vyletělová et al. (2011) showed phenotypic resistant to 30 μg cefoxitin in disc-diffusion method. That kind of differences between phenotype resistance and the presence of genes conferring resistance may be due to the presence of a so-called "silent gene" that is express only *in vivo*, or to the fact that detection by PCR of a single gene inside an operon may overlook the absence of other genes which are necessary for phenotypic expression. Discordances might be brought about by mutations in the coding or promoter region of the PCR-detected genes.

According to the literature data, it is probable that coagulase-negative staphylococci are the source of SCCmec (Leonardo and Markey, 2008). This hypothesis is supported at least by the observed total homology of SCCmec cassettes in the examined coagulase-positive and coagulase-negative staphylococci. The research shows, among others, that the type IV SCCmec cassette, initially determined in *S. epidermidis*, also began to appear over the years in *S. aureus* (Chrobak et al., 2009).

What is significant is the strain resistance to antibiotics of the MLS group (Macrolides–Lincosamides–Streptogramins). 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 (Thumu and Halami, 2012). Macrolide and lincosamide resistance have been observed previously in various CoNS including *S. epidermidis* from cows with mastitis (Sawant et al., 2009). In our study 32.8% revealed multi-drug resistance (MDR) defined as resistance to antibiotics belonging to three or more classes. Two out of the examined strains (belonging to the *S. xylosus* species) revealed simultaneous resistance to antibiotics of nine various classes. Staphylococci are common habitants of skin of warm-blooded animals and the slaughtering process plays an important role for natural contamination of raw material, notably contributing to the bacterial population involved in food of animal origin (Marty et al., 2012).

Tetracycline 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) (Huys et al., 2004). The most frequent gene occurred in our phenotypic tetracycline resistant strains were *tet*(M) following by *tet*(L). *Tet*(K) gene was always associated with *tet*(M) and *tet*(L). This results are in agreement with finding by Bhargava and Zhang (2012) which detected *tet*(M) in 36 of 56 investigated strains. 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 *tet*(L) gene on plasmids and *tet*(M) on conjugative transposons (Tn*916*–Tn*1545* family) contributes to the spread of these determinants. The strains that harboured multiple tetracycline resistant genes were largely isolated from cured meat, only one strain was isolated from cheese. The carriage of multiple *tet* genes was commonly found in individual Gram-positive bacteria (Huys et al., 2004; Rizzotti et al., 2005). Four isolates which were phenotypically tetracycline's susceptible were found to carry at least one of *tet* genes. Seven isolates resistant to erythromycin carried the *msr*(A) gene, which codes for an ATP-dependent efflux pump. This gene occurred in the studied staphylococci more often than other macrolide resistance genes.

5. Conclusions

The analysis of results obtained in this study proves the frequent occurrence of antibiotic-resistant strains among coagulase-negative staphylococci isolated from ready-to-eat food of animal origin. What is particularly alarming is the high percentage of

methicillin resistant strains. Gram-positive bacteria acquire and transfer resistance to antibiotics much more often and more easily than Gram-negative bacteria. The results obtained indicate the need for food monitoring (especially food of animal origin) for the presence of antibiotic-resistant coagulase-negative staphylococci and the possibility of transferring and transmitting antibiotic-resistance genes. Coagulase-negative staphylococci present in ready-to-eat food can pose indirect hazard for consumer health serving as reservoir of resistance genes.

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