Polish Journal of Veterinary Sciences Vol. 15, No. 4 (2012), 677-683

DOI 10.2478/v10181-012-0105-4

Original article

Phenotypic and genotypic antimicrobial resistance of staphylococci from bovine milk

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Abstract

The aim of this study was to examine phenotypic and genotypic antimicrobial resistance of staphylococci from milk samples from cows with subclinical and clinical mastitis and from cows without mastitis symptoms to methicillin, tetracyclines, macrolides and lincosamides (ML). Of 207 strains, including 34 *S. aureus* and 173 coagulase-negative staphylococci (CNS), 11 (6.4%) CNS strains were phenotypically resistant to methicillin. The *mecA* gene was detected by PCR only in two *S. xylosus* strains and one strain of *S. epidermidis* and *S. simulans*. No methicillin-resistant *S. aureus* strains were observed. In methicillin-resistant strains with *mecA,* gene resistance to other investigated antibiotics was not observed. Phenotypic resistance to tetracycline was detected in 11.0% of CNS strains and 47.4% of them carried the *tetK* gene. Of 173 CNS strains studied, 27 (15.6%) were resistant to at least one ML antibiotic. The resistance gene *ermC* was detected in 55.5% of the 27 ML-resistant strains. The *ermA* and *ermB* genes were detected in 14.8% and 11.1% of ML-resistant CNS strains, respectively. Antimicrobial resistance to methicillin, tetracyclines and macrolides was detected more frequently in staphylococcal strains from clinical mastitis compared to animals with subclinical symptoms and without mastitis, while the resistance to lincosamides showed a similar frequency in all groups of cows. In conclusion, CNS species from bovine milk differ in phenotypic and genotypic antimicrobial resistance profiles, and the use of PCR technique alone for the detection of methicillin, macrolide, lincosamide and tetyracycline resistance in CNS from cattle is not reliable.

Key words: *S. aureus*, coagulase-negative staphylococci, antimicrobial resistance, resistance genes, PCR

Introduction

Staphylococci are the predominant pathogen in bovine mastitis (Taponen et al. 2006, Rajala-Schultz et al. 2009, Sampimon et al. 2009a, Sawant et al. 2009).

Staphylococcus aureus is generally coagulase-positive and can cause clinical mastitis, as well as producing persistent subclinical mastitis (Malinowski et al. 2006a, Taponen and Pyorala 2009). Currently, coagulase-negative staphylococci (CNS) are frequently isolated from cows with clinical and subclinical mastitis in many modern dairy herds where efficient mastitis control protocols are observed (Gillespie et al. 2009, Sampimon et al. 2009a, Sawant et al. 2009).

Antimicrobials are commonly used for control of bacteria causing mastitis on dairy farms (Sawant et al. 2005), but resistance to β-lactams, macrolides and lincosamides (ML antibiotics) have been reported in staphylococci from the milk of cows with mastitis (Luthje and Schwarz 2006, Sawant et al. 2009). Tetracyclines are widely used in veterinary medicine, in livestock rearing, in agriculture and in human medicine.

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A number of genes conferring resistance to these groups of antibiotics have been identified in staphylococci. Three determinants, *ermA*, *ermB* and *ermC*, which confer resistance to macrolides, lincosamindes and streptogramins, have been identified (Lina et al. 1999). Tetracycline resistance coded by a wide variety of determinants in different staphylococcal species is frequently encountered (Trzcinski et al. 2000). The spread of antibiotic resistance among CNS species in cattle may represent a hazard for human health through transfer of resistance genes between staphylococcal species, including *S. aureus*, and through direct transmission of resistant pathogens between humans and animals (Walther and Perreten 2007).

The aim of this study was to determine phenotypic and genotypic antimicrobial resistance of staphylococci from milk of cows with and without mastitis to antibiotics.

Materials and Methods

Quarter milk samples

Quarter milk samples from cows with subclinical and clinical mastitis from twenty seven herds were collected in the central part of Poland between February 2009 and March 2010. Quarter milk samples for microbiological analysis were also collected from cows without mastitis symptoms for comparison purposes. The diagnosis of mastitis was made on the basis of clinical examination of the udder by the veterinarian. Additionally, a quarter was identified as infected when somatic cell counts (SCC) were above 200 000/ml (Casadevall and Pirofski 2000, Malinowski et al. 2006b). SCC were measured with Fossomatic™Minor (Foss, Denmark). The quarter milk samples were collected aseptically by scientific personnel of the Department of Microbiology or by field veterinary surgeons. Before sample collection, the cow teats were cleaned and dipped in an approved disinfectant. The teat ends were then sanitized with swabs containing 70% isopropyl alcohol and allowed to dry. The first few streams were discarded, and 10 ml of milk was then collected aseptically into sterile vials. Milk samples were cooled and immediately transported to the laboratory.

Bacterial identification

A total of 71 staphylococcal isolates from milk of cows with clinical mastitis and 73 isolates from milk of cows with subclinical mastitis, as well as 63 isolates from milk samples from cows without mastitis symptoms, were used in this study.

Preliminary identification of isolates was based on colony and microscopic morphology, catalase test, coagulase testing with rabbit plasma and DNase activity. Coagulase-positive staphylococci were streaked on peptone agar (p-agar) (BBL, Becton Dickinson, Sparks, Md.) supplemented with 7 mg/l of acriflavin (Sigma-Aldrich, Steinheim, Germany), and incubated at 37°C for 24 hours. Bacterial growth in the full length of the streak on p-agar was considered confirmative of *S. aureus* (Capurro et al. 1999).

The presumptive isolates as CNS in the first stage of identification were tested based on conventional microbiological procedures which include haemolysis patterns and use of biochemical profile (Bannerman 2003). Biochemical characteristics of isolates included evaluation of ability to utilize the sugars: xylose, arabinose, sucrose, trehalose, maltose, mannitol, lactose, xylitol, ribose, fructose, and mannose; nitrate reduction, presence of urease and ornithine decarboxylase, and resistance to novobiocin (5 μg) characterized by inhibition zones measuring ≤ 16 mm in diameter.

Finally, all isolates were identified to the species level with an API Staph ID 32 system (bioMerieux, Lyon, France). Apiweb software (bioMerieux) was used to determine the probability of species identification. Identification with a probability ≥ 90% was considered acceptable (Taponen et al. 2006, Sampimon et al. 2009b). Isolates for which the reliability of identification was <90% were then subjected to analysis with the VITEK GPI card system (VITEK 2 instrument, version 4.01, bioMérieux).

The isolates were stored at -80°C in Brain Heart Infusion Broth (BHI; BBL, Becton Dickinson) with 15% glycerol.

Determination of phenotypic antimicrobial resistance

Antibiotic resistances of staphylococci were tested with a disc diffusion method according to the Clinical and Laboratory Standards Institute (2011). The following antibiotic discs (Oxoid, Basingstoke, UK) were used: erythromycin (15 μg), clindamycin (2 μg), and tetracycline (30 μg). Cefoxitin (30 μg) was used for detection of methicillin resistance of isolates (Broekema et al. 2009). *S. aureus* ATCC 29213 and *S. aureus* ATCC 25923 were used as control strains to ensure accuracy of testing.

DNA isolation

Genomic DNA was isolated from resistant staphylococci using the IT 1-2-3™ DNA Sample

Table 1. Primers and PCR conditions.

Target genes	Primer sequences (5' → 3')	Amplicon length (bp)	Reference	No. of PCR cycles (conditions)
16SrDNA	cagctcgtgtcgtgagatgt (F) aatcatttgtcccaccttcg (R)	420	Strommenger et al. 2003	30 (2 min at 95°C; 1 min at 54°C; 1 min at 72°C)
mecA	aaaatcgatggtaaaggttggc (F) agttctgcagtaccggatttgc (R)	533	Merlino et al. 2002	35 (30 s at 94°C; 30 s at 55°C; 1 min at 72°C)
tet K	gtagcgacaataggtaatagt(F) gtagtgacaataaacctccta(R)	360	Strommenger et al. 2003	30 (30 s at 95°C; 30 s at 55°C; 30 s at 72°C)
tetM	agtggagcgattacagaa(F) catatgtcctggcgtgtcta(R)	158	Strommenger et al. 2003	Same as for *tetK*
ermA	gttcaagaacaatcaatacagag(F) ggatcaggaaaaggacattttac(R)	421	Leclercq et al. 1989	30 (30 s at 94°C; 30 s at 55°C; 1 min at 72°C)
ermB	ccgtttacgaaattggaacaggtaaagggc(F) gaatcgagacttgagtgtgc(R)	359	Trieu-Cuot et al. 1990	30 (30 s at 94°C; 30 s at 57°C; 1 min at 72°C)
ermC	gctaatattgtttaaatcgtcaattcc(F) ggatcaggaaaaggacattttac(R)	572	Leclercq et al. 1989	Same as for *ermA*

(F) – forward primers, (R) – reverse primers.

Purification Kit (Idaho Technology Inc., Salt Lake City, Utah, USA) according to the manufacturer's protocol. 2.5 µl of the total extracted material from each test sample was used as a template DNA for PCR application.

Detection of antibiotic resistance genes

The presence of the *mecA* gene responsible for resistance to β-lactam antibiotics, the *tetK* and *tetM* genes responsible for tetracycline resistance, and the *ermA*, *ermB*, *ermC* genes responsible for macrolide and lincosamide resistance were investigated by PCR. Primers amplifying a specific staphylococcal 16SrDNA region were used for genotypic confirmation of isolates belonging to *Staphylococcus*. The specific primers and the PCR conditions are shown in Table 1. The primers were synthesized by DNA-Gdańsk (Poland).

PCR was carried out on the staphylococcal isolates displaying phenotypic resistance to at least one of the antibiotics. Amplification of DNA was performed using the Multi Gene II thermal cycler (Labnet International, Inc., USA).

A duplex PCR was used to detect the presence of the *tetK* and *tetM* genes simultaneously. The PCR mixture was performed in a 25-µl volume containing 2.5 µl of DNA template, 1 x PCR buffer, 0.2 mM each of dATP, dCTP, dGTP, and dTTP (Fermentas, Lithuania), 200 nM each of *tetK* and *tetM* pair of primers, and 1 U of RedTag Genomic DNA polymerase (Sigma-Aldrich, Germany). A duplex PCR was also used to detect the presence of the *ermA* and *ermC* genes. The components of the PCR mixture with 200 nM each of *ermA* and *ermC* primers were the same as those for the *tetK* and *tetM* genes. The size of the amplified fragment was 533 bp for the *mecA* gene. DNA samples were amplified in a total of 25 µl of the following reaction mixture: 1 x PCR buffer, 200 µM of each dATP, dCTP, dGTP, dTTP (Fermentas), 200 nM *mecA* primers, and 1U RedTag Genomic DNA polymerase (Sigma-Aldrich). The PCR mixture in 25 µl total volume was used for detection of the *ermB* gene at final concentrations of 200 nM *ermB* primers and for detection of 16SrDNA at final concentrations of 100 nM 16SrDNA primers. Remaining reaction components were the same as those for the *mecA* gene.

A negative control with each of the reaction components except for template DNA, positive control with the genomic DNA from *S. aureus* for the *mecA* gene, positive control with genomic DNA from *S. xylosus* for the *ermA*, *ermB*, *ermC* genes, positive control with genomic DNA from *S. xylosus* for the *tetK* gene and positive control with genomic DNA from *Staphylococcus spp*. for the *tetM* gene, were included with each test run. The PCR products were analyzed by electrophoresis in 1.5% agarose gels stained with ethidium bromide. Molecular size markers (GenoPlast Biochemicals, Poland, and Sigma-Aldrich) were also run for product size verification. The gel was electrophoresed in 2 x Tris-borate buffer at 70 V for 1.5 h.

Table 2. Distribution of antibiotic resistance genes among resistant staphylococcal strains isolated from milk of cows with clinical and subclinical mastitis and from cows without mastitis symptoms.

Strains (no.)	Type of mastitis (no.)	No. of resistant strains				Results (no. of strains) of PCR for:					
		FOX	CM	T	E	16S rDNA	*mecA*	*tetK*	*ermA*	*ermB*	*ermC*
S. aureus (34)	Clinical (30)	–	1	1	1	30	–	+(1)	–	–	+(1)
	Subclinical (3)	–	–	–	–	3	–	–	–	–	–
	Without mastitis (1)	–	–	–	–	1	–	–	–	–	–
S. xylosus (91)	Clinical (25)	2	7	3	5	25	+(1)	+(2)	(+)1	(+)1	+(4)
	Subclinical (33)	2	10	3	7	33	+(1)	+(1)	(+)1	(+)1	+(3)
	Without mastitis (33)	3	–	2	1	33	–	+(2)	–	–	+(1)
S. sciuri (36)	Clinical (2)	1	1	1	1	2	–	–	–	–	+(1)
	Subclinical (22)	–	3	3	1	22	–	–	–	–	–
	Without mastitis (12)	–	–	1	–	12	–	+(1)	–	–	–
S. epidermidis (31)	Clinical (6)	1	–	1	1	6	+(1)	+(1)	–	–	–
	Subclinical (14)	1	2	1	1	14	–	–	+(1)	–	+(1)
	Without mastitis (11)	–	1	1	2	11	–	+(1)	–	–	+(1)
S. simulans (6)	Clinical (3)	1	–	–	–	3	+(1)	–	–	–	–
	Without mastitis (3)	–	–	–	–	3	–	–	–	–	–
S. lentus (4)	Clinical (3)	–	1	1	1	3	–	+(1)	–	+(1)	–
	Without mastitis (1)	–	–	1	–	1	–	–	–	–	–
S. capre (2)	Clinical (2)	–	1	1	1	2	–	–	–	–	+(1)
S. equorum (3)	Subclinical (1)	–	–	–	1	1	–	–	–	–	+(1)
	Without mastitis (2)	–	1	–	2	2	–	–	+(1)	–	+(2)
Total (207)		11	28	20	25	207	4	10	4	3	16

Results

Resistance to methicillin, tetracyclines, macrolides and/or lincosamides was investigated in 207 strains including 34 *S. aureus* and 173 CNS originating from the milk of cows with clinical or subclinical mastitis and from the milk of cows without mastitis. Affiliation of strains to *Staphylococcus* was confirmed by identification of 420 bp PCR product specific for 16SrDNA (Table 2). Resistance to cefoxitin was detected in 11 (6.4%) CNS strains, among which five strains were isolated from milk samples of cows with clinical mastitis (12.2%), three strains from cases of subclinical mastitis (4.3%) and three strains from milk samples of cows with no signs of mastitis (4.8%). No methicillin-resistant *S. aureus* strains were observed (Table 2). Resistance to tetracycline was detected in 19 (11.0%) of CNS strains. The percentage of tetracycline-resistant CNS strains from milk samples of cows with clinical mastitis was 17.1% and was higher than the percentage of tetracycline-resistant CNS strains from cases of subclinical mastitis (10%) and from the milk of cows without mastitis (8.1%). Resistance to clindamycin and erythromycin (ML antibiotics) was found in one strain of *S. aureus* from clinical mastitis, which was also resistant to tetracycline, and in 27 (15.6%) strains of CNS, which were resistant to at least one ML antibiotic. The percentage of clinadamycin-resistant CNS strains from clinical (24.4%) and subclinical (21.4%) cases of mastitis was similar. Among the most frequently isolated species of CNS were *S. xylosus*, *S. sciuri* and *S. epidermidis* and among of them 18.7%, 11.1% and 9.7% were resistant to clindamycin, respectively. Among 173 of CNS strains 24 (13.9%) showed phenotypic resistance to erythromycin. The percentage of erythromycin-resistant CNS strains from milk samples of cows with clinical mastitis was 22% and was higher than the percentage of erythromycin-resistant CNS strains from cases of subclinical mastitis (14.3%) and from the milk of cows without mastitis (8.1%). All *S. equorum* strains were resistant to erythromycin. In group of *S. xylosus* strains were 14.3% of erythromycin-resistant strains. Phenotypic resistance to methicillin, lincosamides, tetracyclines and macrolides was verified by PCR amplification of the genomic DNA of the resistant strains.

The specific PCR product for the *mecA* gene was detected in only two *S. xylosus* strains and one strain of *S. epidermidis* and *S. simulans* (Table 2, Fig. 1A). In methicillin-resistant strains with the *mecA* gene, resistance to other investigated antibiotics was not observed. The tetracycline resistance gene *tetK* was detected in 50% of tetracycline-resistant strains. Strains

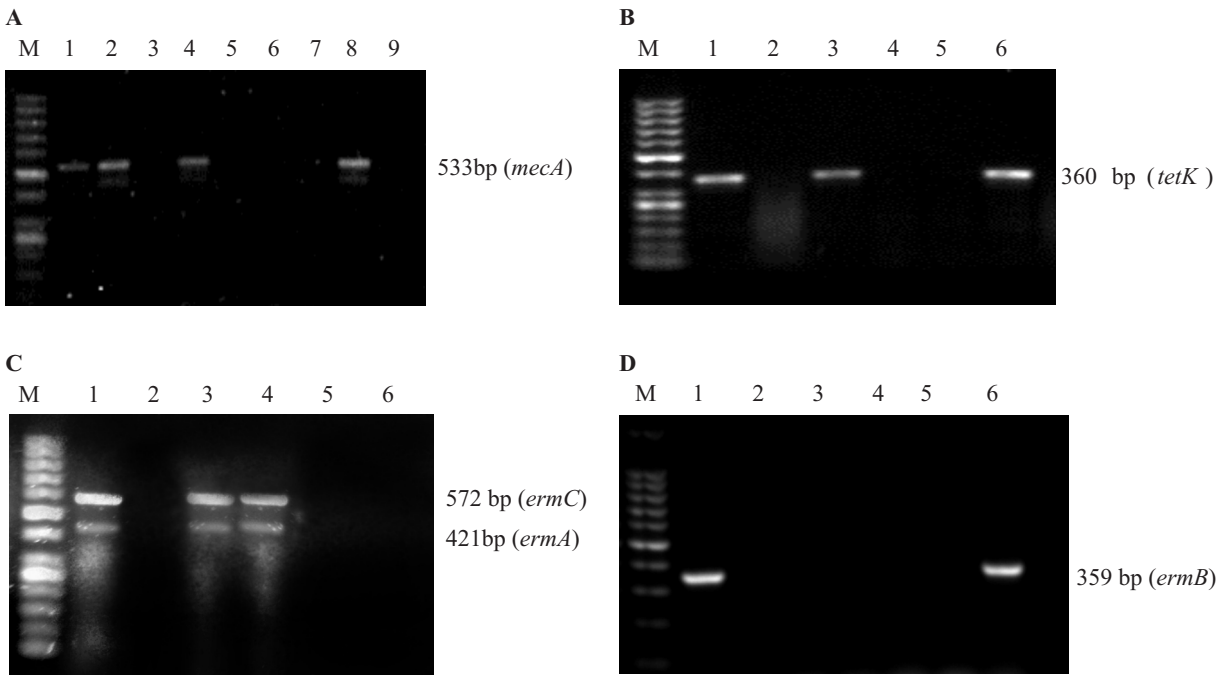

Fig. 1. Electrophoresis in 1.5% agarose gel PCR products obtained by using specific primers for *mecA* gene (A), *tetK* and *tetM* genes (B), *ermA* and *ermC* genes (C), *ermB* gene (D).
A, B, C – Lines M – molecular weight markers (1000, 900, 800, 700, 600, 500, 400, 300, 250, 200, 150, 100, 50 bp – GenoPlast Biochemicals); D – Line M – molecular weight markers (1000, 900, 800, 700, 600, 500, 400, 300, 200, 100 bp – Sigma-Aldrich);
A – Lines: 1 – 533 bp product obtained using genomic DNA of *S. aureus* strain (positive control); 2 – product obtained using genomic DNA of *S. simulans* strain; 3, 5, 6, 7, 9 – no products using genomic DNA of *S. sciuri, S. xylosus, S. epidermidis, S. xylosus* and *Staphylococcus sp.* strains; 4 – product obtained using genomic DNA of *Staphylococcus sp.* strain; 8 – product obtained using genomic DNA of *S. epidermidis* strain.
B – Lines: 1 – 360 bp product obtained using genomic DNA of *S. xylosus* strain (positive control); 2, 4, 5 – no products using genomic DNA of *S. capre, S. lentus, S. sciuri* strains; 3 – product obtained using genomic DNA of *S. xylosus* strain; 6 – product obtained using genomic DNA of *S. sciuri* strain.
C – Lines: 1 – 572 bp and 421 bp products obtained using genomic DNA of *S. xylosus* strain (positive control); 2, 5, 6 – no products using genomic DNA of *S. sciuri*, *S. epidermidis*, *S. lentus* strains; 3, 4 – products obtained using genomic DNA of *S. xylosus* strains.
D – Lines: 1 – 359 bp product obtained using genomic DNA of *S. xylosus* strain (positive control); 2, 3, 4, 5 – no products using genomic DNA of *S. caprae, S. epidermidis, S. sciuri, S. equorum*; 6 – product obtained using genomic DNA of *S. xylosus* strain.

in which the *tetK* gene was detected belonged to the species *S. aureus*, *S. xylosus*, *S. sciuri*, *S. epidermidis* and *S. lentus* (Fig. 1B). Half of the strains with the *tetK* gene came from clinical cases of mastitis.

The most prevalent *erm* gene in CNS strains was *ermC*, which was detected in 55.5% of 27 ML-resistant strains and in one *S. aureus* strain isolated from the milk of a cow with clinical mastitis. The *ermA* and *ermB* genes were seldom detected in CNS strains, because specific products of PCR were obtained for 14.8% and 11.1% of ML-resistant strains, respectively. The *ermA* gene was found in two strains of *S. xylosus* and one strain of *S. epidermidis* and *S. equorum*, whereas the *ermB* was detected in two strains of *S. xylosus* and one strain of *S. lentus* (Table 2). Specific PCR products for *ermA*, *ermB* and *ermC* are shown in Fig. 1C and D. The genotype of multi resistant *S. aureus* was *tetK*+*ermC*. The combination of *tetK*+ *ermA*+*ermB*+*ermC* was noted as a separate genotype in two *S. xylosus* strains and the *tetK*+*ermC* genotype was observed in one *S. xylosus* strain. The *ermA*+*ermC* genotype was detected in one strain of *S. epidermidis* and *S. equorum*. The *ermC* gene alone was detected in 10 CNS strains. The *tetK* gene alone was identified in 6 tetracycline-resistant CNS strains, and with other resistance genes in 4 CNS strains.

Discussion

In our study we investigated the distribution of genetic resistance determinants in staphylococcal strains from the milk of cows with and without mastitis which were methicillin-resistant or resistant to at least one of the antibiotics such as clindamycin, tetracycline and erythromycin. Methicillin-resistant

staphylococci are considered resistant to β-lactams which are important antimicrobial agents used for the prevention and treatment of mastitis in dairy cows (Sawant et al. 2009). In this study the *mecA* gene encoding PBP2a (penicillin-binding protein) was detected only in four of 11 phenotypic methicillin-resistant NS strains. Tenover et al. (1999) also observed *mecA*-negative CNS strains which were classified as methicillin-resistant by both the MIC and disc diffusion method. Resistance in these isolates may be due to changes in penicillin binding proteins (PBPs) other than PBP2. Changes in PBPs1 and 4 in methicillin-resistant and *mecA*-negative *S. haemolyticus* and *S. saprophyticus* strains were reported by Suzuki et al. (1992). York et al. (1996) showed discrepancies between the results of phenotypic methicillin resistance and *mecA* results, particularly in *S. saprophyticus*. In our study, the *mecA* gene was not detected in five *S. xylosus* strains, and in one *S. sciuri* and *S. epidermidis* strain, which showed phenotypic methicillin resistance. Martin et al. (2006) reported the presence of the *mecA* gene in only 3.6% of 194 *S. xylosus* and none of 11 *S. carnosus* strains. Resch et al. (2008) did not detect the *mecA* gene in any of the phenotypically resistant CNS strains associated with food using PCR and specific primers. One of 12 *S. epidermidis* strains from bovine milk which was resistant to methicillin did not carry *mecA* (Sawant et al. 2009). It indicates that detection of methicillin resistance in CNS strains could not be limited only to a search for the *mecA* gene because absence of this gene does not confirm susceptibility of CNS strains to β-lactams. In our study we did not detect other resistance genes in methicillin-resistant staphylococci.

Macrolide and lincosamide resistance have been observed in various CNS from cows with mastitis (Lüthje and Schwarz 2006). Macrolide-lincosamide resistance in staphylococci is mainly based on the dimethylation of an adenine residue in the 23S rRNA (Werckenthin et al. 1999). Among the methylase genes (*ermA*, *B*, *C* and *F*), the plasmid-encoded gene *ermC* is most widely distributed in human and animal staphylococci (Lina et al. 1999, Khan et al. 2002, Simeoni et al. 2008). In our study *ermC* was detected in one *S. aureus* strain from the milk of cows with clinical mastitis and in 55.5% of the ML-resistant strains. The highest percentage (75.8%) of staphylococci isolates with the *ermC* gene, from an entire swine production chain, was obtained by Simeoni et al. (2008). In our study we detected *ermA* and *ermB* genes in 14.8% and 11.1% of ML-resistant CNS strains, respectively. The *ermA* gene was also detected by Simeoni et al. (2008) in a low number of staphylococcal isolates from the food production chain, but the *ermB* gene was detected in 56.1% of isolates.

In this study, among 20 strains resistant to tetracycline 50% carried the *tetK* gene and in none of them was the *tetM* gene detected. The *tetK* gene is located on small multicopy plasmids and *tetM* on conjugative transposons (Chopra and Roberts 2001) which may be lost as a result of the passage of strains on the culture media, which may explain the lack of specific PCR products for these genes. High incidence of *tetK* and *tetM* genes was observed in staphylococci from the food production chain (Simeoni et al. 2008). The widespread occurrence of the *tetK* gene in food-associated CNS was reported by Resch et al. (2008), who simultaneously did not detect the *tetM* gene in any tetracycline-resistant strains using PCR and specific primers.

In our study, phenotypic antimicrobial resistance of CNS strains to methicillin, tetracyclines and macrolides was detected more frequently in strains isolated from cases of clinical mastitis compared to isolates from subclinical cases and from healthy cows, while the percentage of lincosamide-resistant CNS strains was similar in isolates from clinical and subclinical mastitis. Malinowski et al. (2002) showed that staphylococcal strains isolated from clinical mastitis did not differ in their sensitivity from strains isolated from subclinical mastitis.

Antimicrobial therapy using β-lactams, lincosamides, macrolides or tetracyclines is not always effective due to the prevalence of antibiotic resistance in various CNS which cause mastitis in cows. Therefore, the evaluation of antimicrobial susceptibility of individual CNS species is necessary before antimicrobial therapy of mastitis caused by these microorganisms. The PCR technique, however, is not sufficient to confirm the antimicrobial susceptibility of CNS.

Occurrence of antibiotic resistance in various CNS, which in the last two decades have emerged as significant pathogens both in human and veterinary medicine, may cause problems due to the risk of horizontal transfer of antibiotic resistance determinants to commensals or pathogenic bacteria.

References

Bannerman TL (**2003**) Staphylococcus, micrococcus, and other catalase-positive cocci that grow aerobically. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH (eds) Manual of clinical microbiology. American Society Microbiology, Washington, pp 384-404.

Broekema NM, Van TT, Monson TA, Marshall SA, Warshauer DM (**2009**) Comparison of cefoxitin and oxacillin disc diffusion methods for detection of *mecA*-mediated resistance in *Staphylococcus aureus* in a large-scale study. J Clin Microbiol 47: 217-219.

Capurro A, Concha C, Nilsson L, Östensson K (**1999**) Identification of coagulase-positive staphylococci isolated from bovine milk. Acta Vet Scand 40: 315-321.

Casadevall A, Pirofski LA (**2000**) Host-pathogen interactions: basic concepts of microbial commensalism, colonization, infection and disease. Infect Immun 68: 6511-6518.

Chopra I, Roberts M (**2001**) Tetracycline antibiotics: mode of action, applications, molecular biology and epidemiology of bacterial resistance. Microbiol Mol Biol Rev 65: 232-260.

Clinical and Laboratory Standards Institute (CLSI) (**2011**) Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. CLSI, Wayne, M100-S21, 31 No. 1.

Gillespie BE, Headrick SI, Boonyayatra S, Oliver SP (**2009**) Prevalence and persistence of coagulase-negative *Staphylococcus* species in three dairy research herds. Vet Microbiol 134: 65-72.

Khan AA, Nawaz MS, Khan SA, Steele R (**2002**) Detection and characterization of erythromycin-resistant methylase genes in Gram-positive bacteria isolated from poultry litter. Appl Microbiol Biotechnol 59: 377-381.

Leclercq R, Bauduret F, Soussy CJ (**1989**) Selection of constitutive mutants of gram-positive cocci inducible resistant to macrolides, lincosamides and streptogramins (MLS): comparison of the selective effects of the MLS. Pathol Biol (Paris) 37: 568-572.

Lina G, Quaglia A, Reverdy ME, Leclercq R, Vandenesch F, Etienne J (**1999**) Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. Antimicrob Agents Chemother 43: 1062-1066.

Lüthje P, Schwarz S (**2006**) Antimicrobial resistance of coagulase-negative staphylococci from bovine subclinical mastitis with particular reference to macrolide-lincosamide resistance phenotypes and genotypes. J Antimicrob Chemother 57: 966-969.

Malinowski E, Kłossowska A, Kaczmarowski M, Lassa H, Kuźma K (**2002**) Antimicrobial susceptibility of staphylococci isolated from affected with mastitis cows. Bull Vet Inst Pulawy 46: 289-294.

Malinowski E, Lassa H, Kłossowska A, Smulski S, Markiewicz H, Kaczmarowski M (**2006a**) Etiological agents of dairy cows' mastitis in western part of Poland. Pol J Vet Sci 9: 191-194.

Malinowski E, Lassa H, Kłossowska A, Markiewicz H, Kaczmarowski M, Smulski S (**2006b**) Relationship between mastitis agents and somatic cell count in foremilk samples. Bull Vet Inst Pulawy 50: 349-352.

Martin B, Garriga M, Hugas M, Bover-Cid S. Veciana-Nogués MT, Aymerich T (**2006**) Molecular, technological and safety characterization of Gram-positive catalase-positive cocci from slightly fermented sausages. Int J Food Microbiol 107: 148-158.

Merlino J, Watson J, Rose B, Beard-Pegler M, Gottlieb T, Bradbury R, Harbour C (**2002**) Detection and expression of methicillin/oxacillin resistance in multidrug-resistant and non- multidrug-resistant *Staphylococcus aureus* in Central Sydney, Australia. J Antimicrob Chemother 49: 793-801.

Rajala-Schultz PJ, Torres AH, Degraves FJ, Gebreyes WA, Patchanee P (**2009**) Antimicrobial resistance and genotypic characterization of coagulase-negative staphylococci over the dry period. Vet Microbiol 134: 55-64.

Resch M, Nagel V, Hertel C (**2008**) Antibiotic resistance of coagulase-negative staphylococci associated with food and used in starter cultures. Int J Food Microbiol 127: 99-104.

Sampimon OC, Barkema HW, Berends IM, Sol J, Lam TJ (**2009a**) Prevalence and herd-level risk factors for intramammary infection with coagulase-negative staphylococci in Dutch dairy herds. Vet Microbiol 134: 37-44.

Sampimon OC, Zadoks RN, De Vliegher S, Supré K, Haesebrouck F, Barkema HW, Sol J, Lam TJ (**2009b**) Performance of API Staph ID 32 and Staph-Zym for identification of coagulase-negative staphylococci isolated from bovine milk samples. Vet Microbiol 136: 300-305.

Sawant AA, Gillespie BE, Oliver SP (**2009**) Antimicrobial susceptibility of coagulase-negative *Staphylococcus* species isolated from bovine milk. Vet Microbiol 134: 73-81.

Sawant AA, Sordillo LM, Jayarao BM (**2005**) A survey on antibiotic usage in dairy herds in Pennsylvania. J Dairy Sci 88: 2991-2999.

Simeoni D, Rizzotti L, Cocconcelli P, Gazzola S, Dellaglio F, Torriani S (**2008**) Antibiotic resistance genes and identification of staphylococci collected from the production chain of swine meat commodities. Food Microbiol 25: 196-201.

Strommenger B, Kettlitz C, Werner G, Witte W (**2003**) Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. J Clin Microbiol 41: 4089-4094.

Suzuki E, Hiramatsu K, Yokota T (**1992**) Survey of methicillin-resistant clinical strains of coagulase-negative staphylococci for *mecA* gene distribution. Antimicrob Agents Chemother 36: 429-434.

Taponen S, Pyörälä S (**2009**) Coagulase-negative staphylococci as cause of bovine mastitis-not so different from *Staphylococcus aureus*? Vet Microbiol 134: 29-36.

Taponen S, Simojoki H, Haveri M, Larsen HD, Pyörälä S (**2006**) Clinical characteristics and persistence of bovine mastitis caused by different species of coagulase-negative staphylococci identified with API or AFLP. Vet Microbiol 115: 199-207.

Tenover FC, Jones RN, Swenson JM, Zimmer B, McAllister S, Jorgensen JH (**1999**) Methods for improved detection of oxacillin resistance in coagulase-negative staphylococci: results of a multicenter study. J Clin Microbiol 37: 4051-4058.

Trieu-Cuot P, Poyart-Salmeron C, Carlier C, Courvalin P (**1990**) Nucleotide sequence of the erythromycin resistance gene of the conjugative transposon Tn*1545*. Nucleic Acids Res 18: 3660.

Trzcinski K, Cooper BS, Hryniewicz W, Dowson CG (**2000**) Expression of resistance to tetracyclines in strains of methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 45: 763-770.

Walther C, Perreten V (**2007**) Methicillin-resistant *Staphylococcus epidermidis* in organic milk production. J Dairy Sci 90: 5351.

Werckenthin C, Schwarz S, Westh H (**1999**) Structural alterations in the translational attenuator of constitutively expressed *ermC* genes. Antimicrob Agents Chemother 43: 1681-1685.

York MK, Gibbs L, Chehab F, Brooks GF (**1996**) Comparison of PCR detection of *mecA* with standard susceptibility testing methods to determine methicillin resistance in coagulase-negative staphylococci. J Clin Microbiol 34: 249-253.