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RESEARCH ARTICLE

Evaluation of Antimicrobial Resistance in *Staphylococcus* Spp. Isolated from Subclinical Mastitis in Cows

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ABSTRACT

The objective of this study was to determine the antibiotic resistance in different *Staphylococcus* isolates using conventional and molecular methods. A total of 61 subclinical mastitis isolates of *Staphylococci* were evaluated for oxacillin, erythromycin, tetracycline, nitrocefin, and cefoxitin using the Kirby-Bauer disc diffusion method. The same isolates were also subjected to the multiplex PCR technique to detect *mec*A, *fem*A and *erm*A, *erm*C, *tet*K, and *tet*M genes. Of the isolates, (*Staphylococcus aureus*, n=34 and coagulase negative *Staphylococcus* (CoNS), n=27) 26, 29, and 8 were resistant to erythromycin, tetracycline, and oxacillin, respectively in phenotypical evaluation. The genotypical evaluation indicated that of the strains, 34 carried *erm* genes in erythromycin-resistant strains and 10 carried *tet* genes in tetracycline-resistant strains. Agreement rates between genotypic and phenotypic evaluation for erythromycin, tetracycline and methicillin were 57, 65.5 and 89% respectively. Data suggest that phenotypical methods should be accompanied by genotypical methods to establish antibacterial resistance accurately, which would enhance treatment efficiency.

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INTRODUCTION

Mastitis negatively affects milk production, milk quality and the economic sustainability of dairy farming throughout the world. The control of bovine mastitis is of paramount importance in dairy animals and its incidence can be reduced by identification of different pathogens and enforcement of effective monitoring system. Establish of mastitis control programs includes various approaches such as dry cow therapy, prevention of infection transmission, improvement of the immune system and treatment of subclinical and clinical cases, are imperative to limit infections and risk factors in dairy herds (Hussain *et al*., 2013). *S. aureus* and CoNS are the most prevalent mastitis pathogens in dairy cows and heifers (Bastan *et al*., 2010). A common intramammary infection (IMI) caused by *S. aureus* that spread expeditiously in dairy herd becomes persistent during lactation. Although, CoNS are considered as a part of normal flora of the udder however, the bacteria cause infections due to lack of local immune response (Simojoki, 2011). Moreover, *S. aureus* and CoNS can develop resistance to antibiotics and become a reservoir for resistance genes in population (Turutoglu *et al*., 2009). Additionally, the transmission of antibiotic resistance among *Staphylococcus* strains and hosts has also been a serious concern (Sawant *et al*., 2009; Hussain *et al*., 2012).

The gene structures have active role in transmission of antibiotic resistance among bacterial species. For example, erythromycin resistance develops by transmission of resistance structures in plasmids or methylation of 23 SrRNA (Leclercq, 2002). The tetracycline resistance genes, which are carried by plasmids and transposons, provides tetracycline resistance, whereas penicillin bound protein (PBP), which is encoded by the *mec*A gene, and excessive production of beta-lactamase cause methicillin resistance in bacteria (Ardıc *et al*., 2005). When excessive and inappropriate antibiotic are used in dairy herds ultimately support the bacterial resistance and becomes a threat not only for animals but also public health.

The aim of this study was to determine the antibiotic resistance profile for erythromycin, tetracycline and methicillin using molecular and conventional methods in

S. aureus and CoNS strains isolated from subclinical mastitis in cows.

MATERIALS AND METHODS

Herd and detection of subclinical mastitis: This study was carried on Brown Swiss cows which were housed in Atatürk University Research Farm and regularly controlled for subclinical mastitis using California Mastitis Test (CMT). CMT positive cows were taken to the study and milk samples from infected mammary quarters were collected aseptically to detect causative pathogen. The procedure described by National Mastitis Council (NMC) (1991) was followed during the aseptic sampling.

Bacterial isolation: Each milk sample (10 µl) was inoculated in agar containing 5% sheep blood and incubated aerobically for 24-48 h at 37°C. *Staphylococcus* species (n=61) growth was identified on the basis of culture and morphological features and by gram staining according to described by Quinn *et al*. (2002). For nitrocefin sensitivity, the manufacturer's recommended protocol was followed (Oxoid-Beta lactamase-BR66A). Isolated and identified strains were stored at -20°C in tryptic soy broth with 15% glycerol until the antimicrobial and molecular analyses.

Antimicrobial susceptibility and beta lactamase activity: Antimicrobial susceptibility for erythromycin, tetracycline, oxacillin, cefoxitin was determined using the Kirby-Bauer disc diffusion method in Mueller-Hinton agar according to standards described by Clinical Laboratory Standards Institute (CLSI, 2013). Mueller-Hinton agar plates were overlaid with an inoculum (turbidity equivalent to that of a 0.5 McFarland Standard) of the *Staphylococcus* spp. Antibiotic discs were applied and incubated 35±2°C for 24 h. Beta-lactamase activity of oxacillin-resistant strains was evaluated using nitrocefin discs (BD BBL Becton, Dickinson and Company USA) (Pitkala *et al*., 2007).

Molecular detection of antimicrobial resistance

DNA isolation and multiplex PCR procedure: For DNA extraction, specimens (count cells of interest [10^6 to 10^7] were suspended in 100 µl of PBS and boiled at 95°C for 15 min and then centrifuged 15,000 rpm for 5 min. Following to centrifugation, supernatant was used as DNA samples. The obtained DNA samples were stored at -20°C until the PCR procedure. Multiplex PCR was performed for both genotyping confirmations of staphylococcal strains by 16SrDNA, *fem*A, and detection of antibiotic resistance by *mec*A, *tet*K, *tet*M, *erm*C and *erm*A genes (Ardıc *et al*., 2005) (Table 1).

For *mec*A and *fem*A genes, 0.4 µM of primers, 200 µM of dNTP, 3 mM of $MgCl_2$ and 2 µl of DNA were added into 25 µl of PCR mix. Reaction mixtures were heated to 95°C for 1 min and were then subjected to 30 cycles of denaturation for 2 min at 95°C, annealing for 1 min at 54°C, extension for 7 min at 72°C, and final polymerization for 7 min at 72°C. For *tet*K, *tet*M, *erm*C *and erm*A genes, 0.4 µM of primer, 0.4 µM of dNTP, 3 mM of $MgCl_2$, and 1.25 U of taq polymerase were prepared to reach 25 µl of the total volume. First denaturation for 3 min at 95°C was followed by 30 cycles of denaturation for 30 s at 95°C, for 30 s at 54°C, for 30 s at 72°C and final polymerization for 4 min at 72°C. 16SrDNA primers were used as internal control in both multiplex PCR reactions.

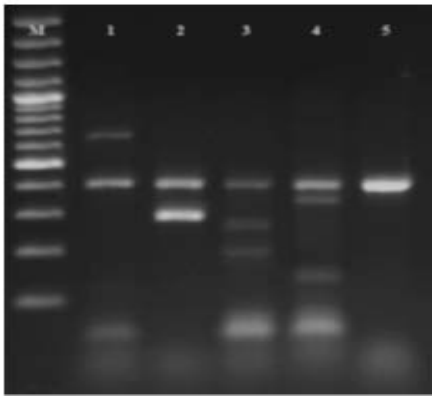

Fig. 1: M: Marker (100 bp DNA Ladder Plus, Fermentas), Lane 1: *S.aureus* (*fem*A: 684bp, 16SrDNA:420 bp), Lane 2: *mec*A positive strain (*mec*A: 314 bp, 16SrDNA: 420 bp), Lane 3: erythromycin positive strain (*erm*A: 190bp, *erm*C: 299bp, 16SrDNA:420 bp), Lane 4: tetracycline positive strain (*tet*K: 360 bp, *tet*M: 158 bp, 16SrDNA: 420 bp)

RESULTS

Antimicrobial susceptibility and beta lactamase activity test results: Of the staphylococcal strains (n=61), 34 were *S. aureus* (55.7%) and 27 were CoNS (44.2%) as presented in table 2. Based on the disc diffusion test in overall evaluation, erythromycin, tetracycline and oxacillin resistance was positive in 26 (42.6%), 29 (47.5%), and 8 (13.1%) of the strains, respectively. In oxacillin-resistant CoNS strains (n=7), 2 were only resistant to cefoxitin, 3 were only resistant to nitrocefin, and 2 were resistant to both nitrocefin and cefoxitin. Resistance to nitrocefin and cefoxitin was not detected in oxacillin resistant *S. aureus* strain (n=1) (Table 2).

Multiplex PCR test results: Specific bands for 420 bp 16SrDNA of *S. aureus* and CoNS were detected in all isolates by PCR. While *fem*A (684bp) was positive in 34 (55.7%) strains, 27 (44.2%) were negative. However, only one CoNS strain showed *mec*A positive result (Fig. 1).

In *S. aureus* strains, 25 (73.5%) were positive for *erm*C gene, while none of the strains had *erm*A. In CoNS strains, 8 (29.6%) were positive for *erm*C, while one of the strain had both *erm*A and *erm*C genes (Table 2). Additionally, *S. aureus* strains had *tet*K (n=2), *tet*M (n=2), and both *tet*K and *tet*M (n=1) genes whereas only *tet*K (n=5) genes were detected in CoNS (Table 2). One strain was positive for both *tet*M and *erm*C genes in *S. aureus* strains, whereas four strains were positive both *tet*K and *erm*C genes in CoNS.

Agreement between genotypic and phenotypic evaluation: Genotypic (PCR results) and phenotypic (antibiogram susceptibility results-disc diffusion method) antibiotic resistance profiles of the strains were given in

Table 1: Oligonucleotide sequences of the primers used in the detection of methicillin resistance

Gene	Primers	Product size (bp)
*mec*A	Forward CCTAGT AAA GCTCCGGAA Reverse CTA GTC CAT TCGGTC CA	314
16SrDNA	Forward CAG CTC GTGTCGTGA GAT GT Reverse AAT CAT TTGTCCCACCTT CG	420
*fem*A	Forward CTT ACT TACTGCTGTACC TG Reverse ATCTCGCTTGTTATGTGC	684
erm(C)	Forward AATCGTCAATTCCTG CAT GT Reverse TAATCGTGGAATACGGGTTTG	299
erm(A)	Forward AAGCGGTAAACCCCTCTG A Reverse TTCGCAAAT CCC TTCTCA AC	190
tet(K)	Forward GTAGCGACA ATA GGTAATAGT Reverse GTAGTGACA ATA AAC CTC CTA	360
tet(M)	Forward AGTGGAGCG ATT ACAGAA Reverse CAT ATGTCCTGGCGTGTC TA	158

Table 2: Conventional and molecular antimicrobial resistance profiles of *S. aureus* and CoNS strains

Resistance	Total	*S. aureus* (n=34)	CoNS (n=27)
Erythromycin	26	13	13
*erm*C	33	25	8
*erm*A	-	-	-
*erm*C+*erm*A	1	-	1
Tetracycline	29	15	14
*tet*K	7	2	5
*tet*M	2	2	-
*tet*K+ *tet*M	1	1	-
Oxacillin	8	1	7
Cefoxitin	2	-	2
Nitrocefin	3	-	3
Nitrocefin+cefoxitin	1	-	1
*mec*A+nitrocefin+cefoxitin	1	-	1

Table 3: Comparison of genotypic (determined by PCR method) and phenotypic (determined by disc diffusion method) antimicrobial resistance profiles of *S. aureus* and CoNS isolates

Antimicrobial resistance		*S. aureus*	CoNS	Total
Erythromycin				
Genotypic (+)	Phenotypic (+)	11	6	17
Genotypic (-)	Phenotypic (-)	7	11	18
Genotypic (+)	Phenotypic (-)	14	3	17
Genotypic (-)	Phenotypic (+)	2	7	9
Tetracycline				
Genotypic (+)	Phenotypic (+)	4	5	9
Genotypic (-)	Phenotypic (-)	18	13	31
Genotypic (+)	Phenotypic (-)	1	-	1
Genotypic (-)	Phenotypic (+)	11	9	20
Methicillin				
Genotypic (+)	Phenotypic (+)	-	1	1
Genotypic (-)	Phenotypic (-)	33	20	53
Genotypic (+)	Phenotypic (-)	-	-	-
Genotypic (-)	Phenotypic (+)	1	6	7

Table 3. Agreement rates, which were either positive or negative results in both genotypic and phenotypic evaluation for erythromycin, tetracycline and methicillin, were 57, 65.5 and 89%, respectively. In terms of isolate, the agreement between genotypic and phenotypic evaluations erythromycin and tetracycline was 53 and 65% for in *S. aureus* strains and 63 and 67% in CoNS strains, respectively.

DISCUSSION

Staphylococcus aureus is one of the most prevalent pathogen in bovine subclinical IMI (Hussain *et al*., 2013). Additionally, these infections sometimes accompanied with CoNS, which are normally obtained in teat end bacterial flora (Simojoki, 2011). Due to excessive and inappropriate antibiotic use against to IMI, these staphylococcal strains develop antibiotic resistance. Especially, multiple resistances to some group of antibiotics such as erythromycin, tetracycline and methicillin can develop in dairy herds and limits antibiotic effectiveness (Simeoni *et al*., 2008).

Although, the rates of multiple-resistant strains have variability between herds and countries, these strains are reported almost in every study (Franca *et al*., 2012). In the current study, multiple-resistant strains were also detected in 5 strains of staphylococcal bacteria (*S. aureus*, n=1; CoNS, n=4). Interestingly, more multiple resistant CoNS than *S. aureus* strains were determined in the study. This result is similar to previous findings regarding CoNS with multiple drug resistance (Simeoni *et al*., 2008; Sawant *et al*., 2009; Kot *et al*., 2012). As a hypotesis, CoNS strains can be a possible reservoir for resistance genes that can be transferred to *S. aureus* (Zmantar *et al*., 2011, Franca *et al*., 2012).

Antimicrobial drug resistance can be determined phenotypically by conventional bacteriological tests or genotypically by molecular tests. In some cases, positivity/negativity by phenotypical and genotypical evaluation may not exhibit agreement (Bhutia *et al*., 2012), suggesting that genes are not the only factors responsible for developing antibiotic resistance develops, especially for erythromycin. In addition to gene functions, efflux pump systems, which provide an antibiotic diluted environment for bacteria, and phosphorylating systems, which are known to inactivate macrolides, are also involved in encouraging erythromycin resistance (Leclercq, 2002).

Previous studies reported that *erm*C is more common than *erm*A in bovine isolates (Ardıc *et al*., 2005; Kot *et al*., 2012) and *erm*A and *erm*C genes are more prevalent in CoNS isolates than in *S. aureus* (Heidari *et al*., 2011; Zmantar *et al*., 2011). In the presented study, *erm*C positivity was compatible with the researchers (Ardıc *et al*., 2005; Kot *et al*., 2012). However, *erm*A and C genes were more prevalent in *S. aureus* strains rather than CoNS.

Differences between genotypic and phenotypic resistance results to erythromycin were also compatible with Countinho *et al*. (2010), who stated phenotypic sensitivity although presence of *erm* genes. In addition, some staphylococcal isolates in the present study were phenotypically resistant to erythromycin despite lacking *erm* genes, which are associated with a lack of *erm* genes in small plasmids (Jaglic *et al*., 2012). According to these results, 1) the erythromycin resistance that is encoded genetically may not be presented phenotypically and these strains may be accepted as potentially erythromycin resistant strains 2) genetically encoding is not essential for presence of phenotypic resistance 3) other assisted resistance developing mechanisms should be taken to the consideration.

Tetracycline resistance is caused by four different resistance genes (*tet*O, *tet*L, *tet*K, *tet*M) located in plasmids (Bismuth *et al*., 1990). *tet*K is the most common, providing plasmid mediated resistance by active efflux. The second most common gene is *tet*M, which is carried by conjugative transposons and protects the bacterial ribosomal structure from tetracycline inactivation (Gao *et al*., 2011). Although tetracycline resistance genes were

more prevalent in CoNS strains than *S. aureus* (Ardıc *et al*., 2005; Kot *et al*. 2012; Simeoni *et al*., 2008) other researchers reported opposite results (Rubin *et al*., 2011; Vyletelova *et al*., 2011). In this study, tetracycline resistance genes were more prevalent in CoNS strains. These variable results can be associated with changing conditions in herds, regions and countries described in previous studies (Franca *et al*., 2012). In contrast with our data, other reports from Turkey (Ardıc *et al*., 2005; Tel and Keskin, 2011) reported a similar distribution of *tet*M and *tet*K in both *S. aureus* and CoNS. According to these results, 1) resistance to tetracycline, which is commonly used antibiotic in treatment of any infection, easily occur due to variety of tetracycline developing mechanism mentioned above, 2) CoNS are quite prone to the development of tetracycline resistance and due to presence in normal flora, 3) CoNS may be primary reservoir for transmission of the *tet* genes. Phenotypically tetracycline-resistant strains were more prevalent than genotypically resistant strains. This might be due to either the lack of evaluation of other genes *tet* (O, L) (Gao *et al*., 2011). It appears that genotypic evaluation to attain resistance to tetracycline is controversial. Additionally, staphylococcal strains, especially in CoNS from mastitic milk have variability in their phenotypic and genotypic antibacterial resistance profiles, and use of PCR method alone for detection of antibacterial resistance in CoNS from mastitic milk may not be reliable (Kot *et al*., 2012)

The presence of methicillin-resistant strains in dairy herds is a risk factor for the emergence and spread of new resistances (Simeoni *et al*., 2008; Febler *et al*., 2010; Bochniarz and Wawron, 2011). This risk is also a threat to cows within an affected herd. Determination of *mec*A gene is accepted as a criterion for detection of genotypic methicillin resistance (Swenson *et al*., 2005). At the same time, cefoxitin and oxacillin must be used together to detect methicillin resistance in order to improve phenotypic specificity. However, the cefoxitin test is accepted as more reliable than oxacillin, which can be affected by incubation temperature and culture medium composition (Simeoni *et al*., 2008; CLSI 2013). In addition to oxacillin resistance, beta-lactamase activity was also evaluated in staphylococcal isolates using nitrocefin as a chromogenic method that determines the existence of methicillin (oxacillin) resistance due to excessive release of beta-lactamase (Pitkala *et al*., 2007).

Briefly, only one CoNS strain, which was also positive for the *mec*A gene, was resistant to both cefoxitin and nitrocefin. The other remaining three CoNS strains were resistant to the cefoxitin. Two of these cefoxitin-resistant strains were neither positive for *mec*A nor positive for nitrocefin (Table 2 and 3). Thus, this result was accepted to be false positive as described before by Broekema *et al*. (2009). As the remaining strain was negative for *mec*A gene, it was positive for nitrocefin that indicated more production of beta lactamase. On the contrary of previous report (Caierao *et al*., 2004), detection of *mec*A resistance gene was rare in the bovine staphylococcal strains as described by Kolar *et al*. (2010). In the current study, the agreement rates in both methods varied between 50 to 65% depend on the antibiotics. Because of this moderate agreement rate between the laboratory methods, resistance mechanisms and previously used antibiotic must be taken to the consideration (Franca *et al*., 2012).

Conclusion: Genotypic evaluation tests depend on genomic antibacterial resistance conditions and phenotypical evaluation tests are sensitive to environmental conditions (i.e. incubation conditions or used methods). According to the findings, detection of genotypic or phenotypic resistance should be evaluated together for diagnose real antibiotic resistance. Additionally, CoNS may be a pool for resistant genes and transfer the genes to the other staphylococcal pathogens in the herd. Therefore, prevalence and antibiotic resistance profile of these strains should also be noticed as with other primary mastitis pathogens.

Author's contribution: SC planned the study. MC examined the cows and detected the subclinical mastitic mammary quarters. SC and GD performed the laboratory procedures. All author wrote, revised and approved the manuscript.

REFERENCES

Ardıc N, M Ozyurt, B Sareyyupoğlu and T Haznedaroğlu, 2005. Investigation of erythromycin and tetracycline resistance genes in methicillin-resistant staphylococci. Int J Antimicrob Agents, 26: 213-218.

Bastan A, M Cengiz, S Cengiz, B Polat, A Colak , M Akan, I Darbaz and DB Acar, 2010. Effects of precalving antibiotic treatment on mastitis and individual somatic cell count in heifers. J Anim Vet Adv, 9: 1245-1249.

Bhutia KO, ST Singh, S Biswas and L Adhikari, 2012. Evaluation of phenotypic with genotypic methods for species identification and detection of methicillin resistant in *Staphylococcus aureus*. Int J App Basic Med Res, 2: 84-91.

Bismuth R, R Zilhao, H Sakamoto, JJ Guesdon and P Courvalin, 1990. Gene heterogeneity for tetracycline resistance in *Staphylococcus* spp. Antimicrob Ag Chemo, 34: 1611-1614.

Broekema NM, TT Van, TA Monson, SA Marshall and DM Warsheuer, 2009. Comparison of cefoxitin and oxacillin disk diffusion methods for detection of *mec*A-mediated resistance in *Staphylococcus aureus* in a large-scale study. J Clin Microbiol, 47: 217–219.

Caierao J, M Musskopf, S Superti, E Roesch, CG Dias and PA D'azeveda, 2004. Evaluation of phenotypic methods for methicillin resistance characterization in coagulase negative Staphylococci (CoNS). J Med Microbiol, 53: 1195-1199.

CLSI, 2013. Performance standards for antimicrobial susceptibility testing; Twenty-third informational supplement, (M100-S23). Wayne, USA, pp: 73-76.

Febler A, C Scott, K Kadlec, R Ehricht, S Monecke and S Schwarz, 2010. Characterization of methicillin-resistant *Staphylococcus aureus* St398 from cases of bovine mastitis. J Antimicrob Chem, 65: 619-25.

Franca CA, RM Peixoto, BM Cavalcante, NF Melo, CJB Oliveira, JL Veschi, RA Mota and MM Costa, 2012. Antimicrobial resistance of *Staphylococcus* spp. from small ruminant mastitis in Brazil. Pesq Vet Brasil, 32: 747-753.

Gao J, M Ferreri, Xq Liu, LB Chen, J Su and B Han, 2011. Development of multiplex polymerase chain reaction assay for rapid detection of *Staphylococcus aureus* and selected antibiotic resistance genes in bovine mastitic milk samples. J Vet Diag Invest, 23: 894-901.

Heidari M, H Momtaz and M Madani, 2011. Detection of the antibiotic resistance genes in *Staphylococcus aureus* isolated from human infections and bovine mastitis. Afr J Microbiol Res, 5: 5132-5136.

Hussain R, MT Javed, A Khan, F Mahmood and R Kausar, 2012. Mastitis and associated histo-pathological consequences in the context of udder morphology. Int J Agric Biol, 14: 947-952.

Hussain R, MT Javed, A Khan and G Muhammad, 2013. Risks factors associated with subclinical mastitis in water buffaloes in Pakistan. Trop Anim Health Prod, 45: 1723-1729.

Jaglic Z, H Vlkova, J Bardon, E Michu, D Cervinkova and V Babak, 2012. Distribution, characterization and genetic bases of erythromycin resistance in staphylococci and enterococci originating from livestock. Zoonoses Public Health, 59: 202–211.

Kolar M, J Bardon, V Hanulik, P Sauer, V Babak and J Sclegelova, 2010. Resistance to methicillin in coagulase negative Staphylococci and its detection. Acta Vet Brno, 79: 261-267.

Kot B, M Piechota, KM Wolska, A Frankowska, E Zdunek, Binek, E Kłopotowska and M Antosiewicz, 2012. Phenotypic and genotypic antimicrobial resistance of staphylococci from bovine milk. Pol J Vet Sci, 15: 677-83.

Leclercq R, 2002. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. Antimicrob Resist, 34: 482-492.

NMC, 1991. National Mastitis Council Lab. Hand Book on Bovine mastitis revised ed. Madison/Wisconsin National Mastitis Council Inc., USA, pp:1-30.

Pitkala A, L Salmikivi, P Bredbacka, AL Myllyniemi and MT Koskinen, 2007. Comparison test for detection of β Lactamase-Producing Staphylococci. J Clin Microbiol, 45: 2031-2033.

Quinn PJ, BK Markey, ME Carter, WJC Donelly, FC Leonard and D Maghire, 2002. Veterinary Microbiology and Microbial Disease. Blackwell Publishing Company, Cornwall, UK.

Rubin JE, KR Ball and M Chirino-Trejo, 2011. Antimicrobial susceptibility of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* isolated from various animals. Can Vet J, 52: 153-157.

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

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

Simojoki H, 2011. Bovine mastitis caused by coagulase negative staphylococci: host response and bacterial factors. Doctorate thesis. Helsinki-Finland.

Swenson JM, FC Tenover and Cefoxitin Disk Study Group, 2005. Results of disk diffusion testing with cefoxitin correlate with presence of *mec*A in *Staphylococcus* spp. J Clin Microbiol, 43: 3818–3823.

Turutoglu H, M Hasoksuz, D Ozturk, M Yıldırım and S Sagnak, 2009. Methicillin and aminoglycoside resistance in *Staphylococcus aureus* isolates from bovine mastitis and sequence analysis of their *mec*A genes. Vet Res Commun, 33: 945-956.

Vyletelova M, O Hanus, R Karpiskova and Z Stastkova, 2011. Occurrence and antimicrobial sensitivity in staphylococci isolated from goat, sheep and cow's milk. Acta Univ Agric Silvic Mendelianae Brun, 59: 209-214.

Zmantar T, B Kouidhi, H Miladi and A Bakhrouf, 2011. Detection of macrolide and disinfectant resistance genes in clinical *Staphylococcus aureus* and coagulase-negative staphylococci. BMC Res Notes, 4: 453.