RESEARCH ARTICLE

ARAŞTIRMA MAKALESİ

Antibiotic resistance of coagulase negative Staphylococci isolated from buffalo milk and some milk products

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SUMMARY

The aim of this study was to determine the antibiotic resistance of coagulase-negative staphylococci (CNS) isolated from raw buffalo milk, tulum cheese and clotted cream in Afyonkarahisar province of Western Turkey. A total of 210 samples were examined between February 2008 and August 2009; of the samples examined 70 were raw buffalo milk, 70 tulum cheeses, and 70 clotted creams. Thirty four isolates (16.2%) obtained from 210 samples were detected as CNS. Of these isolates 12 (35.3%) were recovered from raw buffalo milk, 14 (41.2%) from tulum cheese and 8 (23.5%) from clotted cream. Kirby-Bauer disc diffusion method was used to detect the antibiotic resistance of CNS isolated from samples according to the standards of Clinical Laboratory Standards Institute. Erythromycin was found to be the least effective antibiotic as 79.4% of CNS isolates recovered from raw buffalo milk, tulum cheese and clotted cream samples were resistant to this drug, followed by amikacin (50%), cefalotine (44.1%), penicillin (38.2%), neomycin (35.3%), streptomycin (35.3%), gentamicin (32.4%) and oxacillin (29.4%).

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Manda sütü ve bazı süt ürünlerinden elde edilen koagulaz negatif stafilokokların antibiyotik dirençliliği

SUMMARY

Bu araştırmanın amacı, Türkiye'nin Batı illerinden olan Afyonkarahisar'da kaymak, tulum peyniri ve manda sütünden izole edilen koagulaz negatif stafilokokların antibiyotik direncinin belirlenmesidir. Şubat 2008 ile Ağustos 2009 döneminde 70'i tulum peyniri, 70'i kaymak, 70'i manda sütü olmak üzere toplam 210 örnek incelenmiştir. İncelenen 210 örneğin 34'ünden (%16.2) koagulaz negatif stafilokok izolatı elde edilmiştir. Bu izolatların 12'si (%35.3) manda sütünden, 14'ü (%41.2) tulum peynirinden, 8'i (%23.5) kaymaktan izole edilmiştir. Örneklerden izole edilen koagulaz negatif stafilokokların antibiyotik dirençliliğini belirlemek için CLSI'nin (Clinical Laboratory Standarts Institute) önerdiği Kirby-Bauer Disk Difüzyon metodu kullanıldı. Manda sütü, tulum peyniri ve kaymak örneklerinden elde edilen izolatların %79.4'üne en az etkili antibiyotiğin eritromisin olduğu bulundu. Bunu %50 ile amikasin, %44.1 ile sefalotin, %38.2 ile penisilin, %35.3 ile streptomisin, %32.4 ile gentamisin ve %29.4 ile oksasilin takip etti.

INTRODUCTION

Coagulase-negative staphylococci were isolated from different milk products and raw buffalo milk and screened for their antibiotic resistance.[1-3] CNS are emerging as important mastitis pathogens and can be the cause of substantial economic losses.[4,5] Animals used for food and animal products may serve as a reservoir for resistant bacteria that may be transmitted to human beings. Worldwide concern over the emergence of resistant bacteria in domestic animals and its influence on human medicine is mostly linked to the use of antibiotics as growth promoters, even though some data may be controversial.[6]

Staphylococcus spp. are common commensals on the skin and mucous membranes of humans and warm-blooded animals. Most species are non-pathogenic and may help prevent colonization of the skin by other potential pathogens.[7] They are also isolated from a wide range of foodstuffs such as meat, cheese and milk, and from environmental sources such as soil, sand, air and water. Some species of *Staphylococcus* such as *S. xylosus, S. carnosus* and *S. equorum* are used for flavor and aroma formation during the ripening of fermented foods in the food industry.[8]

Coagulase-negative *Staphylococcus* (CNS) spp. have traditionally been considered to be non-pathogen for a long time. However, this view is changing, because there has been increasing evidence that some CNS species have become predominant pathogens isolated from human and animals in several countries.[4,9-11] It has been reported that some CNS species can present a medical risk. For example *S. saprophyticus*, which may contribute to aroma formation in sausage[12] and help prevent off-flavor during sausage ripening,[13] is an ubiquitous species and is also involved in acute urinary tract infections in young adult women.[10] In recent years, they have also been isolated from animals with subclinical and occasional clinical mastitis and their importance on mastitis has been emphasized in many countries.[4,9, 14-16]

CNS are thought to be a reservoir for resistance genes amplified through antibiotic selection that occurs when antibiotics administered to animals achieve low concentrations on the skin.[17] Resistant bacteria on animals' bodies such as *S. xylosus, S. equorum, S. caprae, S. sciuri* and *S. capitis* may contaminate milk or meat and be subsequently isolated from fermented foods made from raw products. The resistance genes might in some instances be transferred from Staphylococci of animal origin to Staphylococci that cause infections in humans. Thus, antimicrobial treatment may be ineffective for several Staphylococcal infections.[8] In human medicine, antimicrobial multi-resistance is frequently encountered and methicillin-resistant *S. aureus* (MRSA)[18,19] and methicillin-resistant CNS (MR-CNS)[11,20] strains are among the most threatening bacteria involved in nosocomial infections. MRSA as well as multi-resistant *S. aureus* strains and MR-CNS are also reported in veterinary medicine.[4,21] Although penicillinase or β-lactamase resistant penicillins such as methicillin and oxacillin are not used in veterinary medicine in Turkey except for cloxacillin, methicillin-resistant Staphylococci have been important because of their resistance to all other beta-lactam antibiotics.[22,23]

Afyonkarahisar is located in the western region of Turkey and is becoming significant as a breeding area of water buffaloes and dairy cows owing to suitable conditions. The milk and milk products such as tulum cheese and clotted cream obtained from these animals are the sought-after Turkish products. Tulum chese is one of the most popular semi-hard cheeses in Turkey, and is produced from raw sheep, goat or cow milk. In Turkey, although there were several investigations on the antibiotic resistance or susceptibility of CNS isolated from mastitic milk samples,[16,24] the studies on the isolation and antibiotic resistance of CNS isolated from milk products are limited.[25] For this reason, in this study it was aimed to detect the antibiotic resistance of CNS isolated from raw buffalo milk, tulum cheese and clotted cream in Afyonkarahisar.

MATERIALS and METHODS

Collection of Samples

A total of 210 samples were examined between February 2008 and August 2009 in Afyonkarahisar, Turkey. Of the samples examined 70 were raw buffalo milk, 70 tulum cheeses, and 70 clotted creams. Raw buffalo milk samples were obtained from various private milk producers. The other samples were obtained from public bazaars in various regions of Afyonkarahisar. Ten samples of each specimens were collected every month. The samples of each specimens were collected every month. The samples were aseptically transported to laboratory under <10°C.

Isolation of CNS from Samples

Ten grams of each samples were added to 90 mL peptone water and homogenized. An inoculation from each of the dilutions was streaked onto Baird-Parker agar (Oxoid CM 0275) supplemented with egg yolk telluride emulsion (Oxoid SR 0054) and plates were incubated at 37°C for 24 – 48 hours. The moist, smooth, spherical, convex, well-defined contours and grey-black colonies were counted and expressed in CFU/Ml.[26] The five typical colonies were subcultured in blood agar containing 7% sheep blood and the plates were aerobically incubated at 37°C for 24 h. After the incubation, Gram staining, catalase test, glucose oxidation fermentation test and tube coagulase test with rabbit plasma were performed for colonies growing on blood agar. Gram positive, catalase positive and coagulase negative isolates were identified as CNS.[27]

Antimicrobial Susceptibility Test

Kirby-Bauer disc diffusion method was used to detect the antibiotic resistance of CNS isolated from samples according to the standards of Clinical Laboratory Standards Institute **(**CLSI).[28] Pure colonies from the blood agar, incubated 37°C for 18 h, were suspended in 2 Ml sterile saline to a density approximately equal to McFarland opacity No. 0.5. A dry cotton wool swab was placed into the suspension and excess liquid was expressed against the inside of the tube. The bacterial suspension was then inoculated onto Mueller-Hinton agar (Oxoid CM0337) with the swab in such a way that the whole surface of the agar was covered.[28] The plates were incubated at 37°C for 24 h. The results were recorded by measuring the diameter of the zone of inhibition according to the interpretive standards of CLSI.[28]. The following 14 antimicrobial agents were tested: erythromycin (15 µg), neomycin (10 µg), gentamicin (10 µg), tetracycline (10 µg), cefotaxime (30 µg), amikacin (30 µg), cefalotine (30 µg), penicillin (10 µg), oxacillin (1 µg), vancomycin (30 µg), streptomycin (10 µg), ampicillin (10 µg), chloramphenicol (30 µg) and clindamycin (2 µg). Beta lactamase activities of isolates were also examined by using nitrocefin test sticks (Oxoid BR 66 A) as described by the manufacturer.

RESULTS

In the present study, a total of 34 isolates (16.2%) obtained from 210 samples were detected as CNS. Of these isolates 12 (35.3%) were recovered from raw buffalo milk, 14 (41.2%) from tulum cheese, and 8 (23.5%) from clotted cream. A number of isolates recovered from samples and the counts of CNS in the examined food are shown in Table 1.

Thirty four CNS isolates were assayed for antimicrobial resistance using standard disc diffusion test and interpreted via CLSI guidelines. CNS isolates recovered from raw buffalo milk samples showed the highest in vitro resistance rate to erythromycin (75%), followed by neomycin (50%), amikacin (41.7%), cefalotine (33.3%) and oxacillin (33.3%). Similarly, erythromycin was found to be the least effective antibiotic as 92.9% of CNS isolates recovered from tulum cheeses were resistant to this drug, followed by amikacin (64.3%), cefalotine (64.3%), penicillin (64.3%), and streptomycin (50%). The highest in vitro resistance rate for 8 CNS isolates recovered from clotted creams was detected against erythromycin (62.5%), followed by neomycin (37.5%), amikacin (37.5%), gentamicin (37.5%) and streptomycin (37.5%). None of the 8 CNS isolates recovered from clotted creams showed resistance to vancomycin and ampicillin.

The antibiotic resistance rates of CNS isolated from raw buffalo milk, tulum cheese and clotted cream are detailed in Table 2. None of the isolates recovered from samples showed beta lactamase activity.

Table 1. CNS count in the examined food samples
Çizelge 1. *İncelenen gıda örneklerindeki koagülaz negatif stafilokok sayısı*

Samples	No. of samples	No. of isolates (%)	CNS count (cfu/Ml)
Raw buffalo milk	70	12 (35.3)	$7,6x10^3$
Tulum cheese	70	14 (41.2)	$2,2x10^4$
Clotted cream	70	8 (23.5)	$1,0x10^4$
Total	210	34 (16.2)	$1,3x10^4$

Table 2. Results of CNS isolates obtained from food samples
Çizelge 2. Gıda örneklerinden elde edilen koagülaz negatif stafilokokların antibiyotik test sonuçları

Antimicrobial agent	Buffalo milk (n=12 isolates)		Tulum cheese (n=14 isolates)		Clotted cream (n=8 isolates)		Total (n=34 isolates)	
	S	R	S	R	S	R	S	R
	n (%)	n (%)	N (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Erythromycin (15µg)	3 (25)	9 (75)	1 (7.1)	13 (92.9)	3 (37.5)	5 (62.5)	7 (20.6)	27 (79.4)
Neomycin (10µg)	6 (50)	6 (50)	11 (78.6)	3 (21.4)	5 (62.5)	3 (37.5)	22 (64.7)	12 (35.3)
Gentamicin (10µg)	10 (83.3)	2 (16.7)	8 (57.1)	6 (42.9)	5 (62.5)	3 (37.5)	23 (67.6)	11 (32.4)
Tetracycline (10µg)	11 (91.7)	1 (8.3)	8 (57.1)	6 (42.9)	6 (75)	2 (25)	25 (73.5)	9 (26.5)
Cefotaxime (30µg)	11 (91.7)	1 (8.3)	9 (64.3)	5 (35.7)	5 (62.5)	3 (37.5)	25 (73.5)	9 (26.5)
Amikacin (30µg)	7 (58.3)	5 (41.7)	5 (35.7)	9 (64.3)	5 (62.5)	3 (37.5)	17 (50)	17 (50)
Cefalotine (30µg)	8 (66.7)	4 (33.3)	5 (35.7)	9 (64.3)	6 (75)	2 (25)	19 (55.9)	15 (44.1)
Penicillin (10µg)	10 (83.3)	2 (16.7)	5 (35.7)	9 (64.3)	6 (75)	2 (25)	21 (61.8)	13 (38.2)
Oxacillin (1µg)	8 (66.7)	4 (33.3)	10 (71.4)	4 (28.6)	6 (75)	2 (25)	24 (70.6)	10 (29.4)
Vancomycin (30µg)	10 (83.3)	2 (16.7)	12 (85.7)	2 (14.9)	8 (100)	0 (0)	30 (88.2)	4 (11.8)
Streptomycin (10µg)	10 (83.3)	2 (16.7)	7 (50)	7 (50)	5 (62.5)	3 (37.5)	22 (64.7)	12 (35.3)
Ampicillin (10µg)	10 (83.3)	2 (16.7)	11 (78.6)	3 (21.4)	8 (100)	0 (0)	29 (85.3)	5 (14.7)
Chloramphenicol (30µg)	11 (91.7)	1 (8.3)	8 (57.1)	6 (42.9)	6 (75)	2 (25)	25 (73.5)	9 (26.5)
Clindamycin (2µg)	10 (83.3)	2 (16.7)	12 (85.7)	2 (14.9)	7 (87.5)	1 (12.5)	29 (85.3)	5 (14.7)

S: Susceptible , R: Resistant

DISCUSSION

This study investigated the antibiotic resistance of CNS isolated from raw buffalo milk, tulum cheese and clotted cream in Afyonkarahisar. The studies related to isolation of CNS have been focused on mastitic milk samples due to an increase in udder infections caused by CNS recently.[4,15,29,30] The literature often refers to CNS as minor mastitis pathogens,[31] which suggest that CNS are non-pathogenic or mildly pathogenic. However, CNS are frequently isolated as a significant cause of subclinical mastitis and they cause the increase in the somatic cell counts of infected quarters.[32] CNS are also known to cause clinical mastitis and persistent IMI (Intramamary Infections) that can last for several months during lactation in the absence of intervention.[9]

The different isolation rates were reported on CNS species related with mastitis in various countries. Gentilini *et al.*,[4] reported that isolation rate of CNS from 900 bovine milk samples was 13.6% in Argentina. In a study from Germany, 35% of quarters with subclinical mastitis harbored CNS.[15] In Tennessee, USA, the average proportion of CNS infections in high SCC herds was 28%, and herd prevalence ranged from 12% to 41%.[30] The highest prevalence of intramammary infections (IMI) with CNS was reported in Finland, where CNS were isolated from 50% of the quarters positive for bacterial growth in a nationwide survey.[29] In Turkey, the isolation rates of CNS were reported as 20.6% from buffalo milk samples[24] and 20% from bovine milk samples.[33] Despite several investigations on the isolation of CNS from mastitic milk samples, the reported studies on the isolation of CNS from milk products are limited. It was reported by Cunha *et al.*,[34] that a total of 88 different food samples were analyzed and 22.7% were positive for CNS in Brazil. They also reported that isolation rates of CNS from milk and white cheese samples were 10% and 5%, respectively. In a study from Turkey, the isolation rates of CNS were reported as 76.2% from milk and 78.8% from cream samples. In the present study, a total of 34 isolates (16.2%) obtained from 210 samples were detected as CNS. Of these isolates 12 (35.3%) were recovered from raw buffalo milk, 14 (41.2%) from tulum cheese, and 8 (23.5%) from clotted cream. The regional variations and number samples analyzed may be the reason for inconsistent isolation rates.

Several susceptibility or resistance studies have been conducted on CNS isolated from mastitic milk samples.[4,16,2,35] Generally, it was reported that CNS isolates used in these studies showed a high level of resistance to penicillin, erythromycin, ampicillin, cloxacillin and oxacillin. However, the investigations on the antibiotic susceptibility or resistance of CNS isolated from milk products are limited. Some studies showed that Staphylococci from cheese, mostly identified as *S. xylosus* strains, harbored resistance to chloramphenicol, tetracyline, erythromycin and, in lower proportions, to gentamycin, penicillin, lincomycin and kanamycin.[36,37] A study carried out to the antibiotic resistance of CNS associated with food and used in starter cultures, emphasized that resistance to lincomycin, penicillin, fusidic acid, oxacillin, ampicillin and tetracycline were predominant.[38] In our study, erythromycin was found to be the least effective antibiotic as 79.4% of CNS isolates recovered from raw buffalo milk, tulum cheese and clotted cream samples were resistant to this drug, followed by amikacin (50%), cefalotine (44.1%), penicillin (38.2%), neomycin (35.3%), streptomy-

cin (35.3%), gentamicin (32.4%) and oxacillin (29.4%). These results were in agreement with previous studies, especially, in terms of resistance to erythromycin, penicillin, gentamicin and oxacillin. However, the rate of resistance to some antimicrobials showed inconsistency with other similar studies. This may be linked to many factors such as the differences in animal production systems and national policies for the use of antimicrobial drugs in each country. In the present study, the resistance to oxacillin was also remarkable. Although penicillinase or β-lactamase resistant penicillins such as methicillin and oxacillin are not used in veterinary medicine in our country except for cloxacillin, methicillin-resistant Staphylococci have been important because of their resistance to all other beta-lactam antibiotics.[22,23] Some investigations on CNS isolated from different food products showed that some CNS strains produce enterotoxin.[39,3] According to our knowledge, no CNS isolated from milk or dairy products have ever been involved in food poisoning or human pathology cases after ingestion of dairy products.

Nevertheless, a possible clinical significance of some species should not be disregarded in patients with depressed immune systems, long, severe hospital treatments, or in the presence of an indwelling catheter or foreign materials. Some researchers also emphasized that resistance genes might in some instances transfer from Staphylococci of animal origin to Staphylococci that cause infections in humans. Thus, CNS could represent a natural reservoir for antibiotic resistance genes.[17,37,40] The demand on milk and milk products has increased in our region recently owing to suitable conditions for breeding buffalo and dairy cow. Considering the antibiotic resistance and enterotoxin production of some CNS strains isolated from food samples, their importance should not be disregarded for human, especially immunocompromised patients ■

KAYNAKLAR

1. **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 Chemo*, 57: 966-969.
2. **Sawant AA, Gillespie BE, Oliver SP** (2009) Antimicrobial susceptibility of coagulase-negative Staphylococcus species isolated from bovine milk. *Vet Microbiol*, 134: 73-81.
3. **Vernozy-Rozand C, Mazuy C, Prevost G** (1996) Enterotoxin production by coagulase-negative staphylococci isolated from goats' milk and cheese. *Int J Food Microbiol*, 30: 271-280.
4. **Gentilini E, Denamiel G, Betancor A, Rebuelto M, Fermepin RM, De Torres RA** (2002) Antimicrobial susceptibility of coagulase-negative staphylococci isolated from bovine mastitis in Argentina. *J Dairy Sci*, 85: 1913–1917.
5. **Honkakanen-Buzalski T, Myllys V, Pyorala S** (1994) Bovine clinical mastitis due to coagulase-negative staphylococci and their susceptibility to antimicrobials. *J Vet Med B,* 41: 344-350.
6. **Teuber M** (1999) Spread of antibiotic resistance with food-borne pathogens. *Cell Mol Life Sci*, 56: 755-763.
7. **Quinn PJ, Markey BK, Carter ME, Donnelly WJ, Leonard FC** (2002) Veterinary Microbiology and Microbial Disease. Blackwell Publishing Professional, Iowa.
8. **Irlinger F** (2008) Safety assessment of dairy microorganisms: Coagulase-negative staphylococci. *Int J Food Microbiol*, 126: 302-310.
9. **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.
10. **Martineau F, Picard FJ, Ke D, Ménard C, Roy PH, Ouellette M, Bergeron MG** (2000) Development of a rapid PCR assay specific for Staphylococcus saprophyticus and application to direct detection from urine samples. *J Clin Microbiol*, 38: 3280–3284.
11. **Petinaki E, Kontos F, Miriagou V, Maniati M, Hatzi F, Maniatis AN** (2001) The bacterial resistance study group: Survey of methicillin-resistant coagulase negative staphylococci in the hospitals of central Greece. *Int J Antimicrob Agents*, 18: 563- 566.
12. **Samelis J, Mataxopoulos J, Vlassi M** (1998) Stability and safety of traditional greek salami a microbiological ecology study. *Int. J. Food Microbiol.* 44(1-2): 101-117.
13. **Mauriello G, Casaburi A, Blaiotta G, Villani IF** (2004) Isolation and technological properties of coagulase negative staphylococci from fermented sausages of southern Italy. *Meat Sci.* 67: 149-158.
14. **Bedidi-Madani N, Greenland T, Richard Y** (1998) Exoprotein and slime production by coagulase-negative staphylococci isolated from goats milk. *Vet. Microbiol.* 5: 139-145.
15. **Tenhagen BA, Koster G, Wallmann J, Heuwieser W** (2006) Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. *J. Dairy Sci.* 89: 2542 - 2551.
16. **Türütoğlu H, Erçelik S, Öztürk D** (2006) Antibiotic resistance of Staphylococcus aureus and coagulase-negative staphylococci isolated from bovine mastitis. *Bull. Vet. Ins. Pulawy*, 50: 41-45.
17. **Perreten V, Giampa N, Schuler-Schmid U, Teuber M** (1998) Antibiotic resistance genes in coagulase-negative staphylococci isolated from food. *Syst. Appl. Microbiol.* 21(1): 113-120.
18. **Cohen PR, Kurzrock R** (2004) Community-acquired methicillin- resistant Staphylococcus aureus skin infection: an emerging clinical problem. *J. Am. Aca. Dermatol.* 50: 277- 80.
19. **Noguchi N, Nakaminami H, Nishijima S, Kurokawa I, So H, Sasatsu M** (2006) Antimicrobial Agent of Susceptibilities and Antiseptic Resistance Gene Distribution Among Methicilin-Resistant Staphylococcus aureus Isolates from Patients with Impetigo and Staphylococcal Scalded Skin Syndrome. *J. Clin. Microbiol.* 44: 2119-2125.
20. **Agvald-Öhman C, Lund B, Edlund C** (2004) Multiresistant coagulase-negative staphylococci disseminate frequently between intubated patients in a multidisciplinary intensive care unit. *Critical Care.* 8: 42-47.
21. **Van Duijkeren E, Box ATA, Heck MEOC, Wannet WJB, Fluit AC** (2004) Methicillin-resistant staphylococci isolated from animals. *Vet. Microbiol.* 103: 91-97.

22. **Gigure S, Prescott JF, Baggot JD, Walker RD, Dowling PM** (2000) Antimicrobial Therapy in Veterinary Medicine. *Blackwell Publishing. Fourth Edition. USA.*
23. **Lelivre H, Lina G, Jones ME, Olive C, Forey F, Delvallez MR, Chanoine MHN, Bebear CM, Jarlier V, Andremont A, Vandenesch F, Etienne J** (1999) Emergence and spread in French hospitals of methicillin-resistant *Staphylococcus aureus* with increasing susceptibility to gentamicin and other antibiotics. *J. Clin. Microbiol.* 37: 3452-57.
24. **Özenç E, Vural M, Seker E, Uçar M** (2008) An evaluation of subclinical mastitis during lactation in Anatolian buffaloes. *Turk. J. Vet. Ani. Sci.* 32: 359-368.
25. **Vargun F, Vatansever L** (2007) Isolation of staphylococci from milk and cream sold at the Kars market and detection of their enterotoxigenicity. *Medycyna. Wet.* 63: 538-540.
26. **Anon** (2001) Bacteriological Analytical Manual Online. *Food and Drug Administration. Chapter 12.*
27. **Holth JG, Krieg NR, Sneath PHA, Staley JT, Williams ST** (2000) Bergey's Manuel of Determinative Bacteriology, Philadelphia: *Lippincott Williams and Wilkins.*
28. **Clinical Laboratory Standards Institute (CLSI)** (2007) Performance standards for antimicrobial susceptibility testing; *17th informational supplement. CLSI M100- S17. Wayne, PA.*
29. **Pitkälä A, Haveri M, Pyörälä S, Myllys V, Honkanen-1 Buzalskđ T** (2004) Bovine mastitis in Finland 2001—prevalence, distribution of bacteria, and antimicrobial resistance. *J. Dairy Sci.* 87: 2433–2441.
30. **Roberson JR, Mixon J, Rohrbach B, Holland R** (2006) Etiologic agents associated with high SCC dairy herds. In: Proceedings of the *24th World Buiatrics Congress, Nice, France.*
31. **Davidson TJ, Dohoo IR, Donald AW, Hariharan H, Collins K** (2002) A cohort study of coagulase negative staphylococcal mastitis in selected dairy herds in Prince Edward Island. *Can. J. Vet. Res.* 56: 275-280.
32. **Oliver SP, Lewis MJ, Gillespie BE, Dowlen HH, Jaenicke EC, Roberts PK** (2003) Prepartum antibiotic treatment of heifers: milk production, milk quality and economic benefit. *J. Dairy Sci.* 86: 1187-1193.
33. **Kırkan S, Göksoy EÖ, Kaya O** (2005) Identification and antimicrobial susceptibility of Staphylococcus aureus and coagulase negative staphylococci from bovine mastitis in the Aydın region of Turkey. *Turk. J. Vet. Ani. Sci.* 29: 791-796.
34. **Cunha MLRS, Peresı E, Calsolarđ RAO, Junđor JPA** (2006) Detection of enterotoxins genes in coagulase-negative satphylococci isolated from foods. *Brazil. J. Microbiol.* 37: 70-74.
35. **Bengtsson B, Unnerstad HE, Ekman T, Artursson K, Nđlsson-Öst M, Waller KP.** (2009) Antimicrobial susceptibility of udder pathogens from cases of acute clinical mastitis in dairy cows. *Vet. Microbiol.* 136: 142-149.
36. **Mochetti G, Mauriello G, Villani F** (1997) Differentiation of Staphylococcus strains from Italian sausages by antibiotyping and low frequency restriction fragment analysis of genomic DNA. *Syst. Appl. Microbiol.* 20: 432-438.
37. **Perreten V, Schwartz F, Cresta L, Boeglin M, Dasen G, Teuber M** (1997) Antibiotic resistance spread in food. *Nature.* 389: 502-801.
38. **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.
39. **Rosec JP, Guiraud JP, Dalet C, Richard N** (1997) Enterotoxin production by staphylococci isolated from food in France. *Int. J. Food Microbiol.* 35: 213-221.
40. **Irlinger F, Morvan A, El Solh N, Bergere JL** (1997) Taxonomic characterisation of coagulase negative Staphylococci in ripening flora from traditional french cheeses. *Syst. Appl. Microbiol.* 20: 319-328.