

*Research article***INVESTIGATION ON THE ETIOLOGY OF SUBCLINICAL MASTITIS IN JERSEY AND HYBRID JERSEY DAIRY COWS**

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The aim of this study was to investigate the etiology of subclinical mastitis (SCM) in dairy Jersey cows with the use of bacteriological and molecular identification methods. In the study 121 Jersey and 78 hybrid Jersey cows with SCM were observed in the Samsun district of Turkey. A total of 411 California mastitis test (CMT) positive milk samples from these animals were examined bacteriologically. The prevalence of subclinical mastitis was 54.75% and 67.2% in Jerseys and hybrids, respectively. On bacteriological examination, a total of 92 strains were isolated from 411 milk samples. The most prevalent bacteria were *Staphylococcus* spp. (69.56%). Among them 24 isolates were *Staphylococcus aureus* (26.08%) the other isolates were *Streptococcus dysgalactiae* (23.91%), *Enterococcus* spp. (3.26%) and *Streptococcus agalactiae* (3.26%). All strains were identified with bacteriological culture methods, as well as by Polymerase Chain Reaction (PCR). Gram-negative bacteria were not isolated. In conclusion, the etiology of SCM in full blood and hybrid Jersey dairy cows in Samsun and the prevalence of bacteria were determined. The relatively high prevalence of SCM indicates the potential need for the consideration of some factors contributing to the formation of mastitis (e.g. management) as well as bacterial agents. The present study and further studies may be useful to develop mastitis vaccines by means of providing true vaccine strain sources.

Key words: etiology, Jersey dairy cow, molecular identification, subclinical mastitis

INTRODUCTION

Subclinical mastitis still continues to be a major problem in dairy animals such as cattle, buffalo and ewes due to economic losses to dairy farms all over the world. These economic losses are caused by reduced milk yield, discarded milk, replacement cost, extra labor, costs of treatment, veterinary care and culling [1-6].

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Subclinical mastitis is a disease that is difficult to detect due to the absence of any visible indications either in the milk or in the mammary glands. The diagnosis of SCM is based on the somatic cell count (SCC) and microbiological status of udder quarters [7]. CMT is an advantageous test due to its inexpensiveness and allows the detection of the number of somatic cells in the milk sample indirectly. In addition, it is the only cow-side test giving real-time results for selection of the quarters for subsequent bacteriological examination [8]. Bacteriological culture methods and biochemical tests are used to isolate and identify the bacteria causing SCM [9]. The primary causes of mastitis are bacteria, though it is also caused by non-bacterial pathogens such as viruses, fungi, yeasts, chlamydia and mycoplasmas [10]. Many species of bacteria have been determined as causative agents of mastitis in Turkey. Reported bacteria are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Arcanobacterium pyogenes*, *Escherichia coli*, *Corynebacterium bovis*, *Pasteurella multocida*, *Bacillus subtilis*, *Bacillus cereus* and *Micrococcus* spp. [11]. Identification of bacterial pathogens in the milk from cows with increased SCM is regarded as the definite diagnosis of mastitis and is important for epidemiological studies and disease control. Culture of milk samples from cows with increased SCM may yield no bacteria due to the presence of very low numbers of pathogens when samples are collected. Besides, antibiotic residues and leukocytes might be the cause of negative culture results [12]. Because of these limitations, molecular methods such as Polymerase Chain Reaction (PCR) have been used successfully for the identification of various mastitis pathogens. Furthermore, PCR is a rapid method with high sensitivity and specificity which detects bacteria even in the presence of residual antimicrobials or preservatives in the milk [13-16].

The aim of this study was to determine the etiology of subclinical mastitis (SCM) in Jersey dairy cows using bacteriological and PCR methods as well as to investigate the sensitivity of the isolated bacteria to antimicrobial drugs.

MATERIALS AND METHODS

A total of 337 dairy cattle (221 Jersey and 116 Jersey hybrid) at 25 family and agricultural type farms were investigated from March 2011 to February 2012. Among these cattle 199 (121 Jersey and 78 hybrid Jersey) were diagnosed SCM. California mastitis test positive milk samples (225 and 186 were from Jersey and hybrid Jersey cows, respectively) were examined bacteriologically. Afterwards, suspicious colonies were identified morphologically, microscopically and biochemically. Ethical approval was obtained from the Animal Ethics Committee, University of Ondokuz Mayıs, Samsun, Turkey (Approval Nr. 201001).

California mastitis test and milk sample collection

California mastitis test was used in order to detect the presence of SCM in the field. The procedures and interpretations were performed by traditional methods [17]. Teat

ends were disinfected with cotton swabs with 70% alcohol, allowed to dry and the foremilk samples from quarters were discarded. CMT was performed and approximately 15 ml milk samples of positive quarters were collected in sterile tubes and immediately transported under cold conditions to the laboratory.

Isolation and Identification of Microorganisms

After CMT positive samples were brought to the laboratory, they were homogenized with a vortex mixer; each of them was plated on blood agar enriched with 5% sheep blood and McConcey agar plates. The plates were incubated at 37°C for 24-48 hours. Then, suspicious colonies were evaluated for cultural characteristics (haemolysis, pigmentation), microscopy (Gram staining) and biochemical characteristics (catalase, oxidase, coagulase, aesculin hydrolysis, CAMP tests) [18].

Antibiotic susceptibility test

Antibiotic susceptibilities of the strains isolated from the samples in the study were determined by Kirby-Bauer Disc Diffusion Method according to the National Committee for Clinical Laboratory Standards (NCCLS) (2003) [19]. The antibiotic discs were penicillin G (10 U), vancomycin (30 µg), ampicillin-dicloxacillin (10k µg/1 µg), amoxicillin-clavulonic acid (20 µg/10 µg), teicoplanin (30 µg), neomycin (10 µg), enrofloxacin (5 µg), oxytetracycline (30 µg), spiramycin (100 µg), rifaximin (40 µg), rifaximin-cefacertrile (40 µg/30 µg) and cefaperazone (75 µg). The results were recorded as susceptible (S) or resistant (R).

DNA extraction

DNA for direct PCR was extracted by boiling the intact bacteria. A tissue extraction kit (Invitrogen®) was used for PCR application on milk samples according to the manufacturer's instructions.

Polymerase Chain Reaction (PCR)

PCR protocols were used for bacteria commonly isolated from SCM. To identify *Staphylococcus* spp. by PCR a method by Ciftci et al. [16] was used. Briefly, 5 µl of extracted DNA was added to 25 µl of PCR mixture. This PCR mixture consisted of 1XPCR buffer (50 mM KCl, 20 mM Tris HCl), 5 µl of 25 mM MgCl₂, 3 µl of 10 mM deoxynucleotide triphosphat (dNTP) mixture, 1 µl of 20 µM 16S rRNA (*Staphylococcus* spp. specific) primers and 2U Taq DNA polymerase. Amplification conditions were as follows: Initial denaturation at 94°C for 52 min, followed by 30 cycles of 45 s at 94°C, 45 s at 68°C, 90 s at 72°C and final extension at 72°C for 10 min amplicons were loaded onto 1.5% agarose gel containing 1 µg/ml ethidium bromide and amplified DNA fragments were separated by agarose gel electrophoresis. Bands were visualized under UV transilluminator. Identification of *S. aureus* strains by PCR was performed using a method by Kuzma et al. [13]. Briefly, the total reaction volume was 50 µl

and PCR mixture contained 1.5 mM MgCl₂, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton[®]X-100, 200 mM (each) deoxynucleotide triphosphat, 0.2 mM of primers (Table 1) and 0.625 U Taq polymerase. Amplification conditions were as follows: initial denaturation at 95°C for 10 min, 37 cycles of denaturation at 94°C for 1 s, annealing at 55°C for 30 s, elongation at 72°C 1.5 min and final elongation at 72°C 5 min PCR products were separated and visualized as above. A PCR protocol by Abd El-Razik *et al.* [12] was used to identify *S. dysgalactiae* and *S. agalactiae* strains. All reactions were carried out in a 50 µl volume. Two hundred microliter of extracted DNA, 5 µM primer (Table 1) and 25 µl of Taq PCR Master Mix (Taq PCR Master Mix Kit, Cat no 201443, Qiagen[®]) were brought together in this volume.

Table 1. Primers used for identification of the isolates from cases of SCM by PCR

Target	Primer	Sequence (5'-3')	PCR product (bp)	Source
<i>Staphylococcus</i> spp.	16SrRNA	AACTCTGTATTAGGGAAGAACA	756	Ciftci <i>et al.</i> (2009)
	16SrRNA	CCACCTTCCTCCGGTTTGTCCACC		
<i>S. aureus</i>	Sau327	GGACGACATTAGACGATCA	1318	Abd El-Razik <i>et al.</i> (2010)
	Sau1645	CGGGCACCTATTTTCTATCT		
<i>S. dysgalactiae</i>	Sdy105	AAAGGTGCAACTGCATCACTA	281	Abd El-Razik <i>et al.</i> (2010)
	Sdy386	GTCACATGGTGGATTTTCCA		
<i>S. agalactiae</i>	Sag40	CGCTGAGGTTTGGTGTTTACA	405	Abd El-Razik <i>et al.</i> (2010)
	Sag445	CACCTCTACCAACGTTCTTC		
<i>S. uberis</i>	STRU-UbI	TAAGGAAGACGTTGGTTAAG	330	Moatamedi <i>et al.</i> (2007)
	STRU-UbII	TCCAGTCCCTTAGACCTTCT		
<i>Enterococcus</i> spp.	Ent1	TACTGACAAACCATTTCATGATG	112	Ke <i>et al.</i> (1999)
	Ent2	AACTTCGTCACCAACGCGAAC		
<i>E. Coli</i>	Eco2083	GCTTGACACTGAACATTGAG	662	Abd El-Razik <i>et al.</i> (2010)
	Eco2745	GCACTTATCTCTTCCGCATT		

Amplification conditions were as follows: initial denaturation at 95°C for 2 min, 35 cycles of denaturation at 94°C for 45 s, annealing at 57°C and 60°C for 45 s for *S. dysgalactiae* and *S. agalactiae* primers, respectively, elongation at 72°C for 45 s and final extension at 72°C for 10 min. Amplification products were evaluated as above. The PCR protocol by Moatamedi *et al.* [14] was used to identify *S. uberis*. Briefly, in a 50 µl volume, 5 µl of extracted DNA, 2.5 U Taq polymerase, 0.4 mM of each deoxynucleotide triphosphate, 50 pmol of each primer, 5 µl of 10xPCR buffer (500 mM KCl, 200 mM tris-HCl, pH 8.4), MgCl₂ with optimal concentration were brought together. Amplification conditions were as follows: first denaturation at 94°C for 2 min, 40 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s and final extension at 72°C for

7 min. PCR products were evaluated as above. For identification of *Enterococcus* spp. by PCR, a protocol by Ke et al. [20,21] was used. Briefly, 1 ng of extracted DNA was added to 19 µl of PCR mixture (50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton®X-100, 2.5 mM MgCl₂, 0.2 mM of each *Enterococcus*-specific primers (Ent1 and Ent2; Table 1), 200 mM of each deoxynucleotide triphosphate (Pharmacia Biotech®), 3.3 µg/ml of bovine serum albumin (BSA) (Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada), 0.5 U of Taq polymerase (Promega®). Amplification conditions were as follows: first denaturation at 95°C for 3 min, 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 1 min and final extension at 72°C for 7 min. PCR products were evaluated as above.

Statistics

Data were evaluated with SAS (2009) statistics suited to summarize means, frequencies and standard error of means.

RESULTS

Cultural Identification

Of the 411 milk samples, a total of 92 strains were isolated. Of sixty-four strains (69.56%) identified as *Staphylococcus* spp., 24 strains (26.08%) were identified as *S. aureus* according to their colony morphology (hemolysis, pigment production) and biochemical reactions (coagulase, DNase, mannitol fermentation). Of 92 strains, 25 were (27.17%) *Streptococcus* spp. and three of them (3.26%) were *S. agalactiae* and 22 (23.91%) were *S. dysgalactiae*. Three of all the isolates were identified as *Enterococcus* spp. (3.26%). *S. uberis* was not isolated. Isolated bacterial strains and their percentages are given in Table 2.

Table 2. Distribution of the isolates identified by cultural methods and PCR

Bacteria	Cultural identification	PCR from culture	* PCR from milk
	No (%)	No (%)	No (%)
<i>S. aureus</i>	24 (26.08)	24 (26.08)	24 (5.83)
<i>Staphylococcus</i> spp. other than <i>S. aureus</i>	40 (43.47)	40 (43.47)	40 (9.73)
<i>S. agalactiae</i>	3 (3.26)	3 (3.26)	3 (0.72)
<i>S. dysgalactiae</i>	22 (23.91)	22 (23.91)	22 (5.35)
<i>S. uberis</i>	0 (0)	0 (0)	0 (0)
<i>Enterococcus</i> spp.	3 (3.26)	3 (3.26)	3 (0.72)

*PCR analysis results from 411 milk samples

Antibiotic Susceptibility Test

While all *S. aureus* and other *Staphylococcus* spp. strains were susceptible to rifaximin, rifaximin+cefacetrile, they showed resistance against spiramycin. These strains showed

Table 3. Resistance percentages of the bacteria strains against antibiotics

Antibiotics (conc.)	Staphylococcus spp. (n=40)		S. aureus (n=24)		S. agalactiae (n=3)		S. dysgalactiae (n=22)		Enterococcus spp. (n=3)	
	n	%	n	%	n	%	n	%	n	%
Penicillin (10 U)	35	87.5	24	100	3	100	20	90.9	3	100
Vancomycin (30 µg)	27	67.5	18	75	3	100	22	100	3	100
Ampicillin+Dicloxacillin (10 µg+1 µg)	11	27.5	4	16.6	0	0	0	0	0	0
Ampicillin (10 µg)	26	65	22	91.66	0	0	0	0	0	0
Amoxicillin+Clavulanic acid (20 µg+10 µg)	10	25	8	33.33	3	100	22	100	3	100
Spiramycin (100 µg)	40	100	24	100	3	100	22	100	3	100
Teicoplanin (30 µg)	5	12.5	3	12.5	3	100	22	100	3	100
Rifaximin (40 µg)	0	0	0	0	0	0	6	27.27	0	0
Rifaximin+Cefacetrol (40 µg+30 µg)	0	0	0	0	0	0	2	9.09	0	0
Cefepazon (75 µg)	2	3.12	2	8.33	30	100	18	81.81	0	0
Neomycin (10 µg)	35	87.5	24	100	3	100	3	100	3	100
Oxytetracycline (30 µg)	21	52.5	16	66.66	3	100	3	100	3	100

a variety of resistance against other antibiotics. Namely, >50% of the *Staphylococcus* spp. strains (other than *S. aureus*) showed resistance to penicillin (87.5%), neomycin (87.5%), vancomycin (67.5%), ampicillin (65%) and oxytetracycline (52.5%). While all *S. aureus* strains were resistant to spiramycin, penicillin and neomycin, 91.66% of them showed resistance to ampicillin. Besides, *S. aureus* strains showed relatively high resistance to vancomycin (75%) and oxytetracycline (66.6%). *S. aureus* and other *Staphylococcus* spp. strains showed lower resistance to ampicillin+dicloxacillin, amoxicillin+clavulanic acid, teicoplanin and cefaperazone. The resistance percentages of the strains to 12 antibiotics are given in Table 3. All *S. agalactiae*, *S. dysgalactiae* and *Enterococcus* spp. strains were resistant to vancomycin, amoxicillin+clavulanic acid, spiramycin, teicoplanin, neomycin and oxytetracycline. In addition, all of these strains were susceptible to ampicillin and ampicillin+dicloxacillin. Penicillin resistance occurred in all *S. agalactiae* and *Enterococcus* spp. strains. Other resistance percentages are given in Table 3.

Molecular Identification

In Table 2, PCR results from both culture and milk (directly) are given. All strains identified by cultural methods were further identified by PCR protocols from culture. After PCR protocols from direct milk samples collected from respective quarters, the same bacterial strains identified both by cultural and culture-PCR methods were identified again. No Gram negative bacteria (including *E. coli*) was found in milk samples neither by cultural nor by molecular methods.

DISCUSSION

Subclinical mastitis is a major problem since it does not present clinical symptoms in dairy cows. The lack of visible symptoms makes it difficult to recognize the infection in udders and long-term effects which occur during the undetected period. Causative organisms might lead to considerable economic losses because of decreased milk production and altered milk composition [14]. In order to treat mastitis effectively and create prevention strategies etiological agents must be determined. Treatment of mastitis relies heavily on the use of antibiotics as the cause is mostly bacterial [4].

The present study was conducted to determine the etiology of subclinical mastitis with bacteriological and molecular methods in Jersey and hybrids, as well as antibiotic resistance of causative bacteria. Mastitis is a multifactorial disease for which more than 250 microbial species, subspecies and serotypes have been isolated and identified as causative agents [5]. These agents are grouped in three categories as contagious, environmental and the others. Many studies have been performed concerning subclinical mastitis in the world and *S. aureus* was reported to be the most important contagious microorganism in cattle [5, 12-14]. Similarly, the studies conducted in Turkey revealed that *S. aureus* was the most common agent [22-25]. The ratio from the previous studies performed at different geographic regions in Turkey was 28.3% in the eastern Anatolia region [22], 39.04% in Elazığ [26], 24.63% in the southeast Anatolia

region [24], 32.5% in Şanlıurfa [27], 28.7% in the central Anatolia region [23]. In the present study performed in the Black sea region of Anatolia, similar ratio of *S. aureus* was detected (26.08%). In a study from Marmara region [24] *S. aureus* isolation rate was found quite lower (4.44%) than in the other regions of Anatolia. Since management is very important in mastitis prevention; diversity of housing conditions is thought to be effective in the etiology of mastitis.

S. agalactiae is reported to be highly contagious and one of the important causes of subclinical infections. It is an obligate bacteria in the bovine mammary gland that can be transmitted to healthy cows via poor milking hygiene [14, 28]. In some studies, the presence of *S. agalactiae* was detected to be between 6.16-10.7% [24, 26, 28]. In the present study the ratio (3,26%) was determined to be lower than the other studies.

Among *Streptococcus* species, *S. uberis* and *S. dysgalactiae* were important species as environmental agents [25]. The most important source of environmental organisms is bedding material. It was reported that moisture in the environment should be decreased to control the environmental microorganisms. Accordingly, clean pastures and environment, dry milking equipment and pre-dipping hygiene are important factors to reduce the exposure of these organisms [5]. Acar *et al.* [25] have reported that 17 (28.81%) *Streptococcus* spp. were isolated from 59 SCM positive milk samples (1 *S. agalactiae*, 8 *S. dysgalactiae*, 2 *S. uberis*, 6 *S. fecalis*). Similarly, Tel *et al.* [27] have also reported that 16 (6.2%) *Streptococcus* spp. isolation was performed from 258 SCM positive milk samples. In the present study, 25 (27.17%) were isolated from 92 bacteria strain (3 *S. agalactiae*, 22 *S. dysgalactiae*). *S. uberis* was not isolated.

E. coli is an environmental mastitis agent similar to *S. uberis* and *S. dysgalactiae* [5, 22, 24]. Although in the present study *E. coli* was not isolated, in the studies carried out in Kars [22], Şanlıurfa [27], Diyarbakır [24], Elazığ [26] *E. coli* was determined as 5.9%, 6.2%, 8.58%, 8.9%, respectively. However, in the study carried out in Kırıkkale [23] the presence of *E. coli* was determined to be very low (0.94%).

Enterococci are the natural flora agent of the gastrointestinal system in humans and animals and reported to be isolated in the dry period [19, 29]. Its ratio might be 6-42% in mastitis cases [28]. In our study it was 3.6%.

Antimicrobial agents are the most frequently used therapeutics for subclinical mastitis cases. A prudent antibiotic therapy improves udder health, decreases the risk of exacerbating clinical mastitis and prevents economic losses. An important factor affecting the success of antibiotic therapy is the resistance of the agents to antibiotics. In several studies, many differences in the resistance against antibiotics by the strains isolated from subclinical mastitis and different geographical regions have been demonstrated [23, 24, 27, 28, 30, 31]. In our study, resistance of all strains against twelve antimicrobials was tested. All *S. aureus*, *S. agalactiae* and *Enterococcus* spp. and also most of other *Staphylococcus* spp. (87.5%) and *S. dysgalactiae* (90.9%) were resistant to penicillin. All *Streptococcus* and *Enterococcus* strains were found resistant against vancomycin, amoxicillin+clavulanic acid, spiramycin, teicoplanin, neomycin

and oxytetracycline. In many reports [23, 24, 27, 30, 31] most of the *Streptococcus* strains, even all of them isolated from bovine subclinical mastitis cases were reported to be sensitive to penicillin. *S. agalactiae*, a group B *Streptococcus* (GBS) was not reported to be resistant to penicillin so far. However, a few reports are available for GBS clinical strains with reduced sensitivity to penicillin [33, 34]. Moreover, in recent times 60 and 100% of resistance to penicillin have been reported in *Streptococcus* spp. strains isolated from subclinical bovine mastitis by Ikiz et al. [24] and Yeşilmen et al. [28], respectively. Although all *S. agalactiae* (3 strains) and 90.9% of *S. dysgalactiae* strains were found to be penicillin resistant, it should be considered that *streptococci* isolated in our study were few in number. Nevertheless, this resistance should not be ignored. Usually intramammary infusion is the most commonly recommended route to treat mastitis cases and one of the antibiotics that has a good distribution into the mammary gland for mastitis treatment is spiramycin [35, 36]. In many studies [23, 25, 37], strains isolated from subclinical mastitis including *Streptococcus* spp., *Staphylococcus* spp. and *Enterococcus* spp. have been found to be sensitive to spiramycin. However, in our study all strains were resistant to spiramycin. While Malinowski et al. [30] have reported that the neomycin resistance rates of *S. agalactiae* and *S. aureus* strains were 80% and 22.7%, respectively; low sensitivity to neomycin both to *S. aureus* (8.33%) and *S. agalactiae* (4%) has been reported by Ikiz et al. [28]. In our study, all *Streptococcus* spp., *Enterococcus* spp. and *S. aureus* strains and most of the other *Staphylococci* (87.5%) were resistant to neomycin. Similarly, all *Streptococcus* spp., *Enterococcus* spp. and most of *S. aureus* (75%) and other *Staphylococci* (67.5%) were resistant to vancomycin. These findings were compatible with the findings reported by Ikiz et al. [28] indicating that the percentage of susceptibility of *S. aureus* and *S. agalactiae* to vancomycin was 8.33% and 8%, respectively. Generally in many studies, [23, 25, 28, 30, 38] it has been reported that *Streptococcus* spp. and *Staphylococcus* spp. isolates were sensitive to amoxicillin and/or amoxicillin clavulanic acid. We found that all *Streptococci* and *Enterococci* were resistant against amoxicillin clavulanic acid but only 33.33% of *S. aureus* and 25% of other staphylococci were resistant to this antibiotic combination. In contrast to our findings, Yeşilmen et al. [24] have reported that all *Streptococci* were sensitive and all *S. aureus* isolates were resistant to amoxicillin clavulanic acid. Susceptibility of *Staphylococci*, *Streptococci* and *Enterococci* to tetracycline/oxytetracycline has been evaluated in many studies and although some researchers [23, 27, 38, 39] have found that the strains isolated from subclinical mastitis in different regions showed varying percentages of sensitivity to tetracycline, in some studies [24, 25, 28, 30] the sensitivity of the strains to tetracycline/oxytetracycline has been showed to be relatively low and even resistant. Ikiz et al. [28] determined only 16% and 16.6% sensitivity of *S. agalactiae* and *S. aureus* to oxytetracycline, respectively. Acar et al. [25] have reported low sensitivity (35.3%) of their isolates to oxytetracycline. Malinowski et al. [30] reported more than 40% resistance to tetracycline in *S. agalactiae* (40.2%), other streptococci (62.3%) and *S. aureus* (41.7%). In our study all *Streptococci* and *Enterococci* and most of *S. aureus* (66.66%) and other *Staphylococci* (52.5%) were resistant to oxytetracycline. These findings were compatible with the results of the studies mentioned above.

The prominent antibiotic and antibiotic combination, to which most bacterial strains were susceptible, were rifaximin and rifaximin+cefacetrile combination. While all staphylococci were detected to be sensitive to these antibiotics, only 6 (27.27%) and 2 (9.09%) *S. dysgalactiae* strains were resistant to rifaximine and rifaximin+cefacetrile combination, respectively. Rifaximin resistance is known to be rare despite concerns about rifaximin resistance of some bacteria (e.g. *Staphylococcus* spp.) in vivo have been raised [40, 41]. On the other hand, it has been reported that the development of bacterial resistance to rifaximin appears to occur with a low frequency in vitro [41]. Several studies evaluated the resistance of mastitis pathogens against rifaximin in vitro. Malinowski *et al.* [30] reported that the resistance of *S. agalactiae* and other streptococci against rifaximin were 17.6% and 25.8% respectively and for coagulase negative staphylococci the percentage of rifaximin resistance was only 2.9%. Idriss *et al.* [42] reported that the percentage of rifaximin resistance of *S. aureus* and *S. agalactiae* were 5.26% and 50%, respectively.

Consequently, the present study revealed that the ratio of contagious (*S. aureus*, *S. agalactiae*) and environmental (*S. dysgalactiae*, *S. uberis*, *Enterococcus* spp.) agents were 29.34% and 70.66%, respectively. High proportions of environmental agents indicate the importance of milking hygiene and dry cow therapy. On the other hand antibiotic susceptibility of mastitis pathogens to antibiotics was variable. In order to achieve the desired effect of antibiotic treatment, the susceptibility status of the strains isolated from mastitis cases in Samsun region should be considered.

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REFERENCES

1. Bradley AJ: Bovine mastitis: An evolving disease. *Vet J*, 164, 117-126, 2002.
2. Ergun Y, Aslantas O, Kirecci E, Sarıbay MK, Ates AT, Ulku A, Demir C: Prevalence and etiology of subclinical mastitis in Awassi dairy ewes in southern Turkey. *Turk J Vet Anim Sci*, 33, 477-483, 2009.
3. Ozenc E, Vural MR, Seker E, Ucar M: An evaluation of subclinical mastitis during lactation in Anatolian buffaloes. *Turk J Vet Anim Sci*, 32, 359-368, 2008.
4. Pellegrino M, Giraudo J, Raspanti C, Odierno L, Bogni C: Efficacy of immunization against bovine mastitis using *Staphylococcus aureus* avirulent mutant vaccine. *Vaccine*, 28, 4523-4528, 2010.
5. Sudhan NA, Sharma N: Mastitis-An important production disease of dairy animals. *SMVS' DAIRY YEAR BOOK*, 72-88, 2010.
6. Trajčev M, Nakov D, Hristov S, Andonov S I, Joksimović-Todorović Mirjana: clinical mastitis in Macedonian dairy herds. *Acta Vet-Beograd*, 63, 63-76, 2013.

7. Makovický P, Makovický P, Nagy M, Rimárová K, Diabelková J: Genetic parameters for somatic cell count, logsc and somatic cell score of breeds: Improved valachian, tsigai, lacaune and their crosses. *Acta Vet-Beograd*, 64, 386-396, 2014.
8. Pyorala S: Indicators of inflammation in diagnosis of mastitis. *Vet Res*, 34, 565-578, 2003.
9. Vigner C, Arora A, Gilmarti N, Welbeck K, O'Kennedy R: Mastitis detection: current trends and future perspectives. *Tib Tech*, 27, 486-493, 2009.
10. Wellenberg GJ, van der Poel WHM, Van Oirschot JT: Viral infections and bovine mastitis: A review. *Vet Microbiol*, 88, 27-45, 2002.
11. Turkyılmaz S, Yıldız O, Oryasın E, Kaynarca S, Bozdoğan B: Molecular identification of bacteria isolated from dairy herds with mastitis. *Kafkas Univ Vet Fak Derg*, 16, 1025-1032, 2010.
12. Abd El-Razik KA, Abdelrahman KA, Ahmed YF, Gomaa AM, Eldebaky HA: Direct identification of major pathogens of the bubaline subclinical mastitis in Egypt using PCR. *J Am Sci*, 10, 652-660, 2010.
13. Kuzma K, Malinowski E, Lassa H, Klossowska A: Specific detection of *Staphylococcus aureus* by PCR in intramammary infection. *B Vet I Pulawy*, 47, 183-190, 2003.
14. Moatamedi H, Seyfiabad Shapouri M, Chorbanpoor M, Jamshidian M, Gooraninejad SA: Polymerase chain reaction based study on the SCM caused by *S. agalactiae*, *S. dysgalactiae* and *S. uberis* in cattle in Ahvaz. *IJVR*, 8, 260-265, 2007.
15. Rajić Savić N, Katić V, Velebit B: Characteristics of coagulase positive staphylococci isolated from milk in cases of subclinical mastitis. *Acta Vet-Beograd*, 64, 115-123, 2014.
16. Cengiz S, Dinç G, Söğüt MÜ: Detection of several virulence properties, antibiotic resistance and phylogenetic relationship in *E. coli* isolates originated from cow mastitis. *Acta Vet-Beograd*, 64, 413-425, 2014.
17. Bastan A: İneklerde Meme Sağlığı ve Sorunları. 124, Kardelen Ofset, Ankara, 2010.
18. Quinn PJ, Carter ME, Markey BK, Carter GR: *Clinical Veterinary Microbiology*. Mosby-Year Book Europe, London, 1994.
19. National Committee for Clinical laboratory Standards: Performance standards for antimicrobial susceptibility testing; fourteenth informational supplement: M2-A8 performance standards for antimicrobial disk susceptibility test; approved Standard. 8th ed. National Committee for Laboratory Standards, Wayne, PA, 2003.
20. Ciftci A, Findik A, Onuk EE, Savasan S: Detection of methicillin resistance and slime factor production of *Staphylococcus aureus* in bovine mastitis. *Braz J Microbiol*, 40, 254-261, 2009.
21. Ke D, Picard FJ, Martineau F, Me´nard C, Roy PH, Ouellette M, Bergeron MGC: Development of a PCR assay for rapid detection of Enterococci. *J Clin Microbiol*, 37, 3497-3503, 1999.
22. Sevinti DA, Sahin M: Determining of β -lactamase activity and resistance against some antibiotics of *Staphylococcus* strains isolated from bovine mastitis. *Vet Bil Derg*, 25, 23-28, 2009.
23. Macun HC, Yagcı IP, Unal N, Kalender H, Sakarya F, Yildirim M: Agent isolation and antibiotic resistance in dairy cows with subclinical mastitis in Kırkkale. *J Fac Vet Med Univ Erciyes*, 8(2): 83-89, 2011.
24. Yesilmen S, Ozyurtlu N, Bademkiran S: The isolation of subclinical mastitis agents and determination of the sensitive antibiotics in dairy cows in Diyarbakır province. *Dicle Univ Vet Fak Derg*, 1, 24-29, 2012.

25. Acar G, Yılmaz E, Solmaz H, Cantekin Z: The isolation of streptococcal agents from cattle with subclinical mastitis in Hatay region and detection of their susceptibilities against some antibiotics. *AVKAE Derg*, 2, 1-5, 2012.
26. Gulcu HB, Ertas HB: Elazığ yöresinde mezbahada kesilen ineklerde mastitisli meme loblarının bakteriyolojik incelenmesi. *Turk J Vet Anim Sci*, 28, 91-94, 2004.
27. Tel OY, Keskin O, Zonturlu AK, Arserim Kaya NB: Şanlıurfa yöresinde subklinik mastitislerin görülme oranı, aerobik bakteri izolasyonu ve duyarlı antibiyotiklerin belirlenmesi. *FU SBVD*, 23, 101-106, 2009.
28. İkiz S, Basaran B, Bingöl EB, Çetin O, Kasıkçı G, Özgür NY, Uçmak M, Yılmaz O, Gündüz MC, Sabuncu A: Presence and antibiotic susceptibility patterns of contagious mastitis agents (*Staphylococcus aureus* and *Streptococcus agalactiae*) isolated from milks of dairy cows with subclinical mastitis, *Turk J Vet Anim Sci*, 37, 569-574, 2013.
29. Cengiz S, Tekin O, Akan M: Molecular typing of *Enterococcus* spp. isolated from cow mastitis, *Vet J Ankara Univ*, 58, 17-20, 2011.
30. Malinowski E, Lassa H, Smulski S, Klossowka A, Kaczmarowski M: Antimicrobial susceptibility of bacteria isolated from cows with mastitis in 2006-2007, *Bull Vet Inst Pulawy*, 52, 565-572, 2008.
31. Persson Y, Nyman AKJ, Grönlund-Andersson U: Etiology and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden. *Acta Vet Scand*, 53(1): 36, 2011.
32. El-Jakee J, Hableel HS, Kandil M, Hassan OF, Khairy EA, Marouf SA: Antibiotic resistance patterns of isolated from mastitic cows and ewes in Egypt, *Global Vet*, 10, 264-270, 2013.
33. Lopardo H: Antimicrobial resistance in β -hemolytic streptococci in Argentina. In, Méndez-Vilas (Ed): *Communicating Research and Educational Topics and Trends in Applied Microbiology*, 794-798, 2007.
34. Jain B, Tewari A, Bhandari BB, Jhala MK: Antibiotic resistance and virulence genes in *Streptococcus agalactiae* isolated from cases of bovine subclinical mastitis. *Vet Arhiv*, 82, 423-432, 2012.
35. Pyorala S: Treatment of mastitis during lactation. *Ir Vet J*, 62(4): 40, 2009.
36. Du Preez JH: Bovine mastitis therapy and why it fails. *JSAVA*, 71, 201-208, 2000.
37. Bakir M, Sabrina R, Toufik M: Antibacterial susceptibility profiles of subclinical mastitis pathogens isolated in Batna and Setif governorates (east of Algeria), *Vet World*, 4, 537-541, 2011.
38. Bulla TR, Rana YS, Sharma A: Comparison of different routes of administration of antibiotic therapy for sub-clinical mastitis in buffaloes. *Haryana Vet*, 44, 86-87, 2005.
39. Ekin IH, Gurtürk K: İneklerde subklinik mastitis olgularından izole edilen streptokokların sero gruplandırılması ve çeşitli biyokimyasal özellikleri üzerine araştırmalar, *YYU Sag Bil Ens Derg*, 4, 21-27, 1998.
40. Kim M, Morales W, Hani AA, Kim S, Kim G, Weitsman S, Chang C, Pimentel M: The effect of rifaximin on gut flora and staphylococcus resistance, *Dig Dis Sci*, 58, 1676-1682, 2013.
41. Valentin T, Leitner E, Rohn A, Zollner-Schwetz I, Hoenigl M, Salzer HJF, Krause R: Rifaximin intake leads to emergence of rifampin-resistant staphylococci, *JINF*, 62, 34-38, 2011.
42. Idriss S, Foltys V, Tancin V, Kirchnerova K, Tancinova D, Zaujec K: Mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Nitra, Slovakia, *Slovak J Anim Sci*, 47, 33-38, 2014.

ISPITIVANJE ETIOLOGIJE SUBKLINIČKOG MASTITISA DŽERZEJ I HIBRID DŽERZEJ MLEČNIH KRAVA

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Cilj ove studije bio je ispitivanje etiologije subkliničkog mastitisa (SCM) mlečnih Jersey krava uz primenu bakterioloških i molekularnih metoda identifikacije. Tokom studije observirano je u Samsun oblasti u Turskoj 121 Jersey i 78 hibrid Jersey krava sa SCM. Ukupno 411 Kalifornija mastitis test (CMT) pozitivnih uzoraka mleka je bakteriološki ispitano. Prevalencija subkliničkog mastitisa bila je 54,75% i 67,20% kod Jersey i hibridnih krava. Tokom bakteriološkog ispitivanja ukupno 92 sojeva je izolovano iz 411 uzoraka mleka. Najčešće bakterije bile su *Staphylococcus* spp. (69,56%). Među njima bili su *Staphylococcus aureus* (26.08%), *Streptococcus dysgalactiae* (23.91%), *Enterococcus* spp. (3.26%) i *Streptococcus agalactiae* (3.26%). Svi sojevi su identifikovani bakteriološkim metodama i PCRom. Gram negativne bakterije nisu izolovane. U zaključku, utvrđena je etiologija SCM kod čistokrvnih i hibridnih Jersey krava u Samsun oblasti kao i prevalencija bakterija. Relativno visoka učestalost SCM ukazuje na potencijanu potrebu razmatranja faktora koji doprinose razvoju mastitisa (npr. menadžment), kao i bakterijskih agenasa. Predstavljena studija, kao i druge studije, mogu doprineti razvoju vakcinalnih sojeva.