TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2013) 37: 569-574
© TÜBİTAK
doi:10.3906/vet-1302-63

Presence and antibiotic susceptibility patterns of contagious mastitis agents (*Staphylococcus aureus* and *Streptococcus agalactiae*) isolated from milks of dairy cows with subclinical mastitis

Serkan İKİZ[1], Beren BAŞARAN[1], Enver Barış BİNGÖL[2,*], Ömer ÇETİN[2], Güven KAŞIKÇI[3], Naciye Yakut ÖZGÜR[1], Melih UÇMAK[3], Özge YILMAZ[3], Mehmet Can GÜNDÜZ[3], Ahmet SABUNCU[3]

[1]Department of Microbiology, Faculty of Veterinary Medicine, İstanbul University, Avcılar, İstanbul, Turkey
[2]Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, İstanbul University, Avcılar, İstanbul, Turkey
[3]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, İstanbul University, Avcılar, İstanbul, Turkey

Received: 26.02.2013 • **Accepted:** 20.03.2013 • **Published Online:** 26.08.2013 • **Printed:** 20.09.2013

Abstract: Mastitis is recognized as one of the most important diseases affecting the dairy industry. The antibiotic susceptibility test is important to achieve accurate treatment in subclinical mastitis. This study was conducted to determine the presence of contagious mastitis agents (*Streptococcus agalactiae* and *Staphylococcus aureus)* in 270 bovine milk samples collected from 132 dairy cows with subclinical mastitis in 15 different dairy farms located in the Marmara Region of Turkey. A total of 256 bacterial pathogens were isolated, of which 25 isolates were identified as *S. agalactiae* and 12 isolates were identified as *S. aureus*. Antibiotic susceptibilities of the isolates were investigated by disk diffusion method. All of the *S. aureus* isolates were resistant to ceftiofur and all of the *S. agalactiae* isolates were resistant to streptomycin. Sensitivity to other antibiotics tested was varied. The antibiotic susceptibility tests should be performed together with the identification of the bacterial agents in order to achieve effective treatment.

Key words: Cows, subclinical mastitis, *Streptococcus agalactiae*, *Staphylococcus aureus*, antibiotic susceptibility

1. Introduction

Bovine mastitis is an inflammatory reaction caused by various types of bacteria that gain entry into the teat canal and mammary gland, and it is the most significant economic drain on the worldwide dairy industry (1). On the basis of habitat of pathogens, mastitis is classified as contagious mastitis and environmental mastitis. Contagious mastitis is caused by contagious bacteria (*Staphylococcus aureus* or *Streptococcus agalactiae*) living on the skin of the teat and inside the udder, but environmental mastitis is caused by environmental pathogens normally found in soil and feed (e.g., *Escherichia coli*, *Streptococcus uberis*, *Klebsiella* spp.) (2).

S. agalactiae is a major contagious pathogen causing bovine subclinical mastitis that can survive for long periods within the mammary gland and can be transmitted to healthy cows through poor milking hygiene (3,4). *S. aureus* is considered to be one of the most common causes of bovine mastitis in the world, which is most frequently subclinical (1).

Medical therapy comprising antibiotics is an important instrument in the scheme of mastitis control. Bovine mastitis is the most costly disease in the dairy industry due to decreased production and milk quality, increased use of medicaments, and veterinary fees (5). Therefore, mastitis has been recognized as a major disease affecting the dairy industry, especially in its subclinical form (6,7). Although clinical mastitis can be detected by visible changes in milk composition (clots, wateriness) and/or mammary gland (redness, pain, and/or swelling) in the farm environment, subclinical mastitis can only be detected by the measurement of inflammatory components and isolation of the pathogens in the milk (6).

Since somatic cell count (SCC) in milk has been considered a superior marker for subclinical mastitis (7), the use of SCC is critical and essential for determining the reduction of the milk yield. Milk can contain both bacterial pathogens and toxins, and its consumption may directly or indirectly increase the risk of foodborne diseases (8).

Rajala-Schultz et al. (9) stated that bovine mastitis is the single most common reason for antimicrobial use in lactating cows. Therefore, antimicrobial resistance patterns of mastitis pathogens have received much interest in recent years.

* Correspondence: bingolb@istanbul.edu.tr

The present study was conducted to determine the presence of major contagious mastitis agents (*S. agalactiae* and *S. aureus*) in dairy cows with subclinical mastitis and investigate the in vitro susceptibility of the isolates to several antimicrobial agents.

2. Materials and methods

In the present study, 348 lactating cows from 15 different smallholder dairy farms located in the Marmara Region of Turkey were examined. In order to determine subclinical mastitis in dairy cows, clinical examination of the udder and screening using the California mastitis test (CMT) were carried out on the farms. CMT-positive milk samples (CMT +, ++, +++) of each examined quarter were suspected as samples of subclinical mastitis. They were carefully collected for further analyses, transported to the laboratory in an ice-cooled box, and analyzed immediately.

Somatic cell counts were performed using a Fossomatic 90 instrument (Foss Electric, Hillerod, Denmark) after heat treatment at 40 °C for 15 min (10).

After that, milk samples were inoculated into nutrient broth with horse sera, onto nutrient agar with 7% sheep blood, and onto MacConkey agar plates. Inoculations onto blood agar plates were duplicated for each sample and incubated in both aerobic and microaerobic conditions. MacConkey agar plates were incubated aerobically, at 37 °C for 24–48 h. Nutrient broths were also incubated microaerobically, at 37 °C for 24–48 h. Gram staining was performed on the cultures and bacteriological methods were used for the identification. The isolates were identified as *S. agalactiae*, belonging to Lancefield's serological group B, by determination of cultural and biochemical properties and serogrouping, and as *S. aureus* by cultural and biochemical properties including coagulase activity (11). *S. agalactiae* and *S. aureus* isolates were investigated for their in vitro susceptibility to 16 antimicrobial agents. The antibiotic susceptibility tests, according to the guidelines from the Clinical and Laboratory Standards Institute (CLSI), were performed to select the convenient antimicrobial agent. Resistance was determined by measurement of inhibition of growth around the antimicrobial disk according to the zone diameter interpretative standards of the CLSI (12).

3. Results

CMT and SCC results of analyzed milk samples are presented in Table 1. Of the samples, 169 (62.59 %) were evaluated as CMT (+), 67 (24.82 %) were CMT (++), and 34 (12.59 %) were CMT (+++). The means of SCC were 237,481, 1,346,234, and 2,213,847, respectively, according to their CMT results.

Submitted milk samples (n = 270) were characterized as no growth (n = 45), contaminated (n = 5), or containing bacterial pathogens (n = 256). Dispersions of the isolates are shown in Table 2 and antibiotic susceptibility test results for *S. agalactiae* (n = 25) and *S. aureus* (n = 12) isolates are explained in Table 3 for 16 antimicrobial agents. None of the *S. aureus* isolates were found to be resistant to methicillin.

4. Discussion

Nowadays, the economic impact of clinical and subclinical mastitis is high in the dairy industry. Losses occur from decreased milk production, treatment and labor costs, veterinary fees, risk of culling or death of the cow, and reduced milk quality and milk price (7,13). Furthermore, low-quality milk can contain pathogens and their toxins, which may hazardous for human health (8).

The quickest and easiest way to identify subclinical intramammary infection in dairy cows is by using defined parameters within either SCC or CMT scores. Somatic cells are always present in milk and increase due to mammary gland infections (8). SCC in healthy cow's milk is between 50,000 and 100,000 cells/mL, and it is considered unhealthy for consumers when it exceeds 200,000 cells/mL (14).

The means of SCC in the present study were within healthy limits for consumers and were recognized as suspected for subclinical mastitis. Similarly, Kaşıkçı et al. (8) determined that the average SCC of milk samples with subclinical mastitis were 249,453, 1,167,058, and 2,108,139, respectively, while Rişvanlı and Kalkan (15) stated that the mean SCCs of the milk samples were 313,001, 559,007, and 1,563,618, respectively, according to their CMT results. Lafi (16) examined a total of 1210 milk samples from 46 flocks near Irbid, Jordan, and observed that 91% (654/719) of them had fewer than 1.00×10^6

Table 1. SCC of milk samples with subclinical mastitis (n = 270).

Characteristic	n	SCC (1000 cells/mL)			
		Min.	Max.	Average	%
CMT (+)	169	20	1400	237	62.59
CMT (++)	67	100	2300	1346	24.82
CMT (+++)	34	1000	2700	2214	12.59

Table 2. Dispersions of the isolates.

Isolates	Number of isolates	Percentage of results (%)
S. aureus	*9*	3.3
S. agalactiae	19	7.03
S. aureus + S. agalactiae	1	0.3
S. aureus + *Candida* spp.	1	0.3
S. aureus + gram-negative bacilli	1	0.3
S. agalactiae + *Candida* spp.	1	0.3
S. agalactiae + gram-negative bacilli	3	1.1
S. agalactiae + *Staphylococcus* spp.*	1	0.3
Other bacteria	184	68.1
No growth	45	16.6
Contaminated**	5	1.8
Total	270	100

*: Not including *S. aureus*
**: Defined as a mixture of at least 3 environmental type organisms without isolation of major mastitis pathogens.

cells/mL; they added that, in 472 milk samples (39.1%), the bacterial culture yielded at least 1 pathogen. Bhutto et al. (17) emphasized that 507 (53%) of 960 subclinical intramammary infection milk samples had $<150 \times 10^3$, $151–250 \times 10^3$, $251–500 \times 10^3$, $501–750 \times 10^3$, and 751 to $>1000 \times 10^3$ SCC, respectively, according to their CMT results.

Subclinical mastitis, which is a hidden form of mammary gland infection, is a very complex disease with numerous causative agents. Contagious pathogens such as *S. aureus* or *S. agalactiae*, which are the most common agents related to subclinical mastitis, are responsible for the strong indicators of the presence of intramammary infections in a herd (1,4). In the present study, *S. aureus* was detected in 4.44% of analyzed milk samples, while *S. agalactiae* was found in 9.25% of milk samples. The lower isolation rate of *S. aureus* than of *S. agalactiae* is contrary to the previous studies reported from Turkey. The rate of *S. agalactiae* isolation was stated as between 3.86% and 18.9%, while the *S. aureus* isolation rate was determined between 13.3% and 47.3% (18–26). Based on the results of the present study, 37 of 256 (14.4%) isolates were identified as contagious mastitis pathogens.

In this study, *S. agalactiae* was found to be streptomycin- (100%), neomycin- (96%), and vancomycin- (92%) resistant, whereas *S. aureus* was detected to be ceftiofur- (100%), ampicillin-, neomycin-, and vancomycin- (91.67%) resistant. The most effective antibiotics against *S. agalactiae* were amoxicillin/clavulanic acid (88%), while *S. aureus* was susceptible to gentamicin (91.66%).

The β-lactams have become the first choice of antimicrobial agents used for treatment of bovine mastitis in Turkey. Penicillin, amoxicillin/clavulanic acid, and ampicillin are the most commonly used agents for treatment of bovine mastitis, because of their low resistance rates and narrow spectra (27). In the present study, 88% of *S. agalactiae* were classified as amoxicillin/clavulanic acid-susceptible, while 91.67% of *S. aureus* was classified as ampicillin-resistant. Similar results were stated by several researchers in Turkey with 77.3% (26) and 64.3% (24) ampicillin resistance to *S. aureus*.

Aminoglycosides are used for prophylactic purposes in dairy animals (28). Researchers also reported that calves are fed with milk replacer, which contains neomycin (9). In this study, higher resistances to neomycin (91.67% and 96%) were observed against *S. aureus* and *S. agalactiae* isolates, respectively. Rato et al. (29) pointed out that among the 108 bovine isolates tested, the highest antimicrobial resistance patterns against *S. agalactiae* were observed for streptomycin and gentamicin (97.2% and 80.6%, respectively). Our findings are similar to the results of the previous study with 100% and 72% antimicrobial resistance against *S. agalactiae* for streptomycin and gentamicin, respectively. Likewise, several studies

Table 3. Percentages of in vitro susceptibility to selected antimicrobial agents for *S. agalactiae* and *S. aureus* isolates.

Antibiotic	Susceptibility rate (%)	
	S. agalactiae	*S. aureus*
Cefoperazone	32	24.95
Gentamicin	28	91.66
Ampicillin/sulbactam	56	66.66
Ampicillin	20	8.33
Amoxicillin/clavulanic acid	*88*	66.66
Amoxicillin	28	16.66
Trimethoprim/sulfamethoxazole	20	58.33
Vancomycin	8	8.33
Neomycin	4	8.33
Penicillin G	40	75
Streptomycin	0	16.66
Enrofloxacin	36	25
Oxytetracycline	16	16.66
Ceftiofur	12	0
Erythromycin	28	33.33
Lincomycin	20	25

conducted previously in Turkey showed similarity with our data about streptomycin resistance in *S. agalactiae* (22,26) but variable resistance to gentamicin *S. aureus* (22,24,26). Aminoglycosides are not the preferred antimicrobial agents for the treatment of streptococcal mastitis because streptococci have inherited resistance to this class of antibiotics (27).

Ceftiofur hydrochloride, a third-generation cephalosporin, is one of the most commonly preferred systemic antimicrobials in lactating cows in a herd. In this study, the highest resistance to ceftiofur (100%) was observed in *S. aureus* isolates. Some authors' reports showed similarity with our data, which pointed out that *S. aureus* strains from bovine intramammary infections were resistant to ceftiofur (30).

Vancomycin is a glycopeptide antibiotic used in the prophylaxis and treatment of infections caused by gram-positive bacteria. Higher vancomycin resistance in *S. agalactiae* (92%) in this study was contrary to results of some authors who reported that none of the streptococcal isolates tested in their studies showed resistance to vancomycin (22,29).

Milk production is indispensable worldwide, and especially in Turkey. Bovine mastitis is one of the most important diseases that threaten public health and the dairy industry. Routine inspections with CMT and SCC are necessary in the diagnosis of mastitis, and isolation of pathogens and antimicrobial susceptibility testing are essential to control the disease and achieve effective therapy. The causative agents and their susceptibilities to antibiotics can be shown to vary by geographical location and time.

Acknowledgment

The present study was supported by Research Fund of İstanbul University, İstanbul, Turkey, project number UDP-27757.

References

1. Behiry AE, Schlenker G, Szabo I, Roesler U. In vitro susceptibility of *Staphylococcus aureus* strains isolated from cows with subclinical mastitis to different antimicrobial agents. J Vet Sci 2012; 13: 153–161.
2. Bhatt VD, Patel MS, Joshi CG, Kunjadia A. Identification and antibiogram of microbes associated with bovine mastitis. Anim Biotechnol 2011; 22: 163–169.
3. McDonald JS. Bovine mastitis: introductory remarks. J Dairy Sci 1979; 62: 117–118.
4. Merl K, Abdulmawjood A, Lammler C, Zschock M. Determination of epidemiological relationships of *Streptococcus agalactiae* isolated from bovine mastitis. FEMS Microbiol Lett 2003; 226: 87–92.
5. Hoblet KH, Schnitkey GD, Arbaugh D, Hogan JS, Smith KL, Schoenberger PS, Todhunter DA, Hueston WD, Pritchard DE, Bowman GL et al. Costs associated with selected preventive practices and with episodes of clinical mastitis in nine herds with low somatic cell counts. J Am Vet Med Assoc 1991; 199: 190–196.
6. Nielen M, Schukken YH, Van De Broek J, Brand A, Deluyker HA, Maatje K. Relations between on-line electrical conductivity and daily milk production on a low somatic cell count farm. J Dairy Sci 1993; 76: 2589–2596.
7. Durr JW, Cue RI, Monardes HG, Moro-Mendez J, Wade KM. Milk losses associated with somatic cell counts per breed, parity and stage of lactation in Canadian dairy cattle. Livestock Sci 2008; 117: 225–232.
8. Kaşıkçı G, Çetin Ö, Bingöl EB, Gündüz MC. Relations between electrical conductivity, somatic cell count, California mastitis test and some quality parameters in the diagnosis of subclinical mastitis in dairy cows. Turk J Vet Anim Sci 2012; 36: 49–55.
9. Rajala-Schultz PJ, Smith KL, Hogan JS, Love BC. Antimicrobial susceptibility of mastitis pathogens from first lactation and older cows. Vet Microbiol 2004; 102: 33–42.
10. Sierra D, Sanchez A, Luengo C, Corrales JC, Morales CT, Contreras A, Gonzalo C. Temperature effects on Fossomatic cell counts in goat milk. Int Dairy J 2006; 16: 385–387.
11. Quinn PJ, Carter ME, Markey BK, Carter GR. Clinical Veterinary Microbiology. London: Harcourt Publishers Limited, 1999.
12. CLSI. Performance Standards for Antimicrobial Disk and Dilution Susceptibility for Bacteria Isolated from Animals; Approved Standard - Second Edition. CLSI document M31-A3, 28: 65–72. Wayne, PA, USA: Clinical and Laboratory Standards Institute, 2008.
13. Nielen M, Deluyker H, Schukken YH, Brand A. Electrical conductivity of milk: measurement, modifiers, and meta analysis of mastitis detection performance. J Dairy Sci 1992; 75: 606–614.
14. Skrzypek R, Wojtowski J, Fahr RD. Factors affecting somatic cell count in cow bulk tank milk - a case study from Poland. J Vet Med 2004; 51: 127–131.
15. Rişvanlı A, Kalkan C. The effect of age and breed on somatic cell count and microbiological isolation rates in milk of dairy cows with subclinical mastitis. YYU Vet Fak Derg 2002; 13: 84–87 (article in Turkish with an abstract in English).
16. Lafi SQ. Use of somatic cell counts and California Mastitis Test results from udder halves milk samples to detect subclinical intramammary infection in Awassi sheep. Small Rum Res 2006; 62: 83–86.
17. Bhutto AL, Murray RD, Woldehiwet Z. California mastitis test scores as indicators of subclinical intra-mammary infections at the end of lactation in dairy cows. Res Vet Sci 2012; 92: 13–17.
18. Ateş M, Erganis O, Çorlu M, Serpek B. Konya yöresindeki mastitisli ineklerden elde edilen süt örneklerinin mikrobiyel florası ve LDH aktivitesi. Turk J Vet Anim Sci 1991; 16: 19–29 (article in Turkish).
19. Muz A, Gülcü HB, Ertaş HB, Kalender H. Mastitisli inek sütlerinden izole edilen mikroorganizmaların identifikasyonları ve antibiyotiklere duyarlılıkları üzerinde bir araştırma. Fırat Univ Vet J Health Sci 1992; 6: 113–121 (article in Turkish).
20. Turutoğlu H, Ateşoğlu A, Salihoğlu H, Öztürk M. Aerobic agents that cause mastitis in dairy cows in the Marmara region. J Pendik Vet Microbiol 1995; 26: 125–137.
21. Şahin M, Çolak A, Otlu S, Aydın F, Genç O, Güler MA, Oral H. Studies on the prevalence of subclinic and clinic mastitis and antibiotic sensitivity in imported simmental cows in Kars district. Kafkas Univ Vet Fak Derg 1997; 3: 49–55 (article in Turkish with an abstract in English).
22. Ak S. Bacterial agents cause contagious and environmental bovine mastitis in Trakya district and their susceptibility to antibiotics. J Fac Vet Med İstanbul Univ 2000; 26: 353–365 (article in Turkish with an abstract in English).
23. Kuyucuoğlu Y, Uçar M. Determination of the subclinical and clinical mastitis rates in dairy cows, of Afyon region and effective antibiotics. J Vet Med Microbiol 2001; 1: 19–24.
24. Turutoğlu H, Mudul S, Pehlivanoğlu F. Antibiotic susceptibility and β-lactamase prevalence for staphylococci isolated from bovine mastitic milk samples. Acta Vet (Beograd) 2002; 52: 337–344.
25. Gülcü HB, Ertaş HB. Bacteriological investigation of udder lobes of cows with mastitis slaughtered in the Elazığ region. Turk J Vet Anim Sci 2004; 28: 91–94 (article in Turkish with an abstract in English).
26. Tel OY, Keskin O, Zonturlu KA, Kaya NBA. Subclinical mastitis prevalence and determination of the antibiotics susceptibility in Şanlıurfa region. Fırat Univ Vet J Health Sci 2009; 23: 101–106 (article in Turkish with an abstract in English).

27. Gianneechini RE, Concha C, Franklin A. Antimicrobial susceptibility of udder pathogens isolated from dairy herds in the West Littoral Region of Uruguay. Acta Vet Scand 2002; 43: 31–41.

28. Prescott JF. Aminoglycosides and aminocyclitols. In: Prescott JF, Bagott JD, Walker RD, editors. Antimicrobial Therapy in Veterinary Medicine. 3rd ed. Ames, IA, USA: Iowa State University Press, 2000, pp. 191–228.

29. Rato MC, Bexiga R, Florindo C, Cavaco LM, Vilela CL, Santos-Sanches I. Antimicrobial resistance and molecular epidemiology of streptococci from bovine mastitis, Vet Microbiol 2013; 161: 286–94.

30. Watts JL, Salmon SA. Activity of selected antimicrobial agents against Strains of *Staphylococcus aureus* isolated from bovine intramammary infections that produce β-lactamase. J Dairy Sci 1997; 80: 788–791.