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Subclinical Mastitis in Dairy Cattle in Kayseri, Türkiye: Bacteriological Analysis and Antibiotic Susceptibilities*

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Abstract: Mastitis is a disease that causes significant economic losses in dairy farms and is caused by many microorganisms and physical trauma. In this study, it was aimed to detect subclinical mastitis (SCM) using the California Mastitis Test (CMT) and bacteriological analysis in dairy cattle and to determine the antibiotic susceptibilities of the obtained isolates. For this purpose, a total of 400 milk samples from 100 cattle were screened with CMT. The CMT-positive samples were subjected to bacteriological analysis. The samples were inoculated onto 7% sheep blood agar and incubated at 37°C for 24-96 h in aerobic/microaerobic/anaerobic atmospheres. Colonies grown were evaluated. The identification of the obtained isolates was performed by phenotypic tests, MALDI-TOF MS, and 16S rRNA gene sequencing. The susceptibilities of the isolates to nine antibiotics were determined by the disk diffusion method. The prevalence of SCM was detected as 25.75% (103/400) for the CMT results. In the bacteriological analysis, 74 (71.8%) of 103 milk samples from 60 CMT-positive animals were detected as positive, and 75 isolates were obtained. The most frequently defined bacteria were coagulase-negative *Staphylococcus* (n=25), *Staphylococcus aureus* (n=17), and *Trueperella pyogenes* (n=8). The highest antibiotic resistance among all isolates was detected to gentamicin (48%) and tetracycline (32%), while *Staphylococcus* spp. isolates were determined to be highest penicillin (45.2%) and gentamicin (38.1%). In conclusion, the current study revealed that there may be a need for dairy farms in this region to develop useful strategies for the treatment/control of SCM, considering the pathogenic bacteria and high antibiotic resistance.
Keywords: Antibiotic susceptibility testing, bacteriological analysis, MALDI-TOF MS, sequencing, subclinical mastitis

Kayseri'deki Süt Sığırlarında Subklinik Mastitis: Bakteriyolojik Analiz ve Antibiyotik Duyarlılıklar

Öz: Mastitis, süt sığırcılığı çiftliklerinde önemli ekonomik kayıplara yol açan ve birçok mikroorganizma ve fiziksel travmanın neden olduğu bir hastalıktır. Bu çalışmada, süt sığırlarında subklinik mastitisin (SKM), California Mastitis Test (CMT) ve bakteriyolojik analiz kullanılarak tespit edilmesi ve elde edilen izolatların antibiyotik duyarlılıklarının belirlenmesi amaçlandı. Bu amaçla, 100 sığırdan alınan toplam 400 süt örneği, CMT ile tarandı. CMT-pozitif örnekler bakteriyolojik analize tabi tutuldu. Örneklerden %7'lik koyun kanlı agarlara ekim yapıldı ve besiyerleri 37°C'de, 24-96 saat, aerobik/mikroaerobik/anaerobik atmosferlerde inkübe edildi. Besiyerlerinde üreyen koloniler değerlendirildi. Elde edilen izolatların tanımlanması fenotipik testler, MALDI-TOF MS ve 16S rRNA sekans analizi ile gerçekleştirildi. İzolatların dokuz antibiyotiğe duyarlılıkları, disk difüzyon yöntemi ile belirlendi. CMT sonuçlarına göre, SKM prevalansı %25.75 (103/400) olarak saptandı. Bakteriyolojik analizde, 60 CMT pozitif hayvana ait 103 süt örneğinin 74'ü (%71.8) pozitif olarak saptandı ve 75 izolat elde edildi. En sık tanımlanan bakteriler koagülaz negatif *Staphylococcus* (n=25), *Staphylococcus aureus* (n=17) ve *Trueperella pyogenes* (n=8) idi. Tüm izolatlar arasında en yüksek antibiyotik direnci gentamisin (%48) ve tetrasikline (%32) karşı tespit edilirken, *Staphylococcus* spp. izolatları arasında en yüksek penisilin (% 45.2) ve gentamisine (%38.1) karşı saptandı. Sonuç olarak, bu çalışma, patojen bakteriler ve yüksek antibiyotik direnci göz önünde bulundurulduğunda, bölgedeki süt çiftliklerinin SKM'nin tedavisi/kontrolü için yararlı stratejiler geliştirmelerine ihtiyaç olabileceğini ortaya koymaktadır.
Anahtar kelimeler: Antibiyotik duyarlılık testi, bakteriyolojik analiz, dizileme, MALDI-TOF MS, subklinik mastitis

Introduction

Mastitis is defined as the inflammation of the mammary glands and causes physical and chemical alterations in the gland tissue and glandular secretions (Kour et al., 2023). It is one of the most important diseases seen in dairy cattle worldwide and is also responsible for huge financial problems, such as treatment costs and losses in milk production/quality (Tommasoni et al., 2023).

Mastitis, according to symptoms, inflammation, and milk quality changes, is divided into two: clinical mas-

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titis (CM) and subclinical mastitis (SCM) (Cao et al., 2023). Clinical mastitis shows several symptoms, such as red-swollen udder, fever, and milk clots, and subdivides into peracute, acute, and subacute based on the inflammation degree (Cheng and Han, 2020). Subclinical mastitis diagnosis is more difficult according to CM, and dairy cattle with SCM might be overlooked since the animals may not present any clinical signs. In addition, SCM increases the somatic (inflammatory) cell count (SCC) in milk and leads to prolonged losses in milk performance, physiological changes, and rises in the therapy cost (Tommasoni et al., 2023; Michira et al., 2023; Cao et al., 2023). The California Mastitis Test (CMT) is a practical, inexpensive, and cow□side test that indirectly determines SCC in milk in SCM. When the SCC in milk increases, the CMT score also increases proportionally. This is associated with the probability and severity of intramammary infection (Alkan et al., 2014).

Mastitis is a disease with multi-etiological features, but many cases are of bacterial origin worldwide. A wide range of bacteria are responsible for bovine mastitis cases. The species primarily isolated in most cases of mastitis are *Staphylococcus aureus*, *Streptococcus* spp. (*Str. agalactiae*, *Str. dysgalactiae* and *Str. uberis*), *Escherichia coli*, *Klebsiella pneumoniae* and *Mycoplasmopsis bovis* (Kasa et al., 2020; Huma et al., 2022; Kour et al., 2023; Rifatbegović et al., 2024). On the other hand, the bacteria show differences in relative prevalence between several geographies (regions/countries). Therefore, the identification of common bacteria is important for the diagnosis of mastitis, appropriate treatment, inhibition of recurrent infections, and successful mastitis control programs (Al-Harbi et al., 2021; Rifatbegović et al., 2024).

The prevention and control programs for mastitis are generally based on antibiotic therapy. However, antimicrobial resistance (AMR) is a rising concern globally because of the extensive empirical use of antimicrobials (Cheng et al., 2019; Al-Harbi et al., 2021). In recent years, the resistance to various antimicrobials (penicillin, amoxicillin, tetracycline, amikacin, gentamicin, erythromycin, piperacillin, ceftazidime, cefquinome, tigecycline, colistin, vancomycin, etc.) has been observed to increase. Furthermore, the drug residue is another threat (Campos et al., 2022). Therefore, the performance of the antibacterial susceptibility test is important to provide the selection of the most appropriate therapy approach in mastitis cases (Cheng et al., 2019; Al-Harbi et al., 2021).

In the present study, we aimed to determine SCM using CMT and bacteriological analysis in four dairy farms and to evaluate the antibiotic susceptibilities of the recovered isolates.

Material and Methods

Milk Samples

The present study analyzed 400 milk samples from 100 dairy cattle during routine CMT screenings to diagnose SCM in 4 dairy farms (25 animals from each) in Kayseri province of Türkiye. CMT-positive milk samples were collected in sterile falcon tubes, transported under cold conditions to Erciyes University, Faculty of Veterinary Medicine, Microbiology Laboratory, and subjected to bacteriological analysis.

California Mastitis Test

The SCM was detected using the CMT and carried out in line with the method previously reported by Bastan (2013). The first two/three streams of milk were discarded, and approximately 2-3 ml of milk samples were milked from each quarter into four cups of the CMT paddle. Afterwards, an equal amount of CMT solution was added to each cup of the CMT paddle, including milk. The solution was provided to interact with the milk by mixing lightly for 15 seconds. The obtained CMT results based on gel formation were categorized as 0 and trace (negative), trace, 1 (weak positive), 2 (evident positive), and 3 (strong positive). Zero and trace of CMT scores were accepted as negative, but 1, 2, and 3 of CMT scores were evaluated as positive for SCM.

Bacteriological Analysis

Ten µL of the milk samples for bacteriological analysis were inoculated on 7% sheep blood agar (Neogen NCM2013A, USA), and the plates were incubated at 37°C in aerobic (for 24-48 h), microaerobic (for 48-72 h) and anaerobic (for 72-96 h) atmospheres. At the end of the incubation period, the colonies grown on the media were evaluated, and the pure cultures were obtained on 7% sheep blood agar.

The identification of the recovered isolates was performed by phenotypic tests, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), and molecular analysis. In the phenotypic identification, Gram staining, motility test, and biochemical tests were used. For MALDI-TOF MS analysis, the sample preparation of the whole cell was done as described by Dieckmann et al. (2008). In addition, Ultraflex II MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, USA) equipped with an all -solid-state SmartBeam^{TM} laser (Nd: YAG laser), operated at 100 Hz in the positive linear mode (100 ns, 25 kV, and 2.2-20 kDa) under the control of the Flex Control software version 3.0 (Bruker Daltonics), was used to determine all mass spectra. According to the manufacturer's specified criteria, the score results of ≥2.00, 1.70-1.99, and <1.70 were assigned as the correct species, low consistency, and no consistency, respectively (Sogawa et al., 2011).

The molecular analysis (16S rRNA gene sequencing) was performed for the identification of the isolates not identified via MALDI-TOF MS. DNA extraction from the recovered isolates was carried out with the classical single-cell lysis buffer (SCLB) protocol (Olah et al., 2006). The universal 27F and 1492R primers were used for amplification and sequence analysis of a near-complete (1465 bp) portion of the 16S rRNA gene region (Lane, 1991). The obtained chromatograms as a result of sequence analysis were evaluated using Finch TV (version V1.4) and aligned. It was identified in the BLAST (Basic Local Alignment Search Tool) rRNA/ITS database of the National Center for Biotechnology Information (NCBI) and deposited in the National Institutes of Health (NIH) GenBank the 16S rRNA gene sequence results.

Results

The Results of CMT

The 103 udder lobes belonging to 60 dairy cattle were found to be positive for SCM. The prevalence of SCM was detected to be 25.75% (103/400). According to CMT results, there are no significant differences between the rates of mastitis in farms (P=0.365) (Table 1).

The Bacteriological Analysis Results

The rate of 71.8% of milk samples (74/103) belonging to 52 dairy cattle (52/60) were found to be positive. There are no significant differences between the rates of mastitis in farms in the bacteriological analysis results (P=0.344) (Table 1).

Table 1. The detailed results of the California Mastitis Test and the bacteriological analysis

Dairy Farm	CMT		Bacteriological analysis	
	No. of dairy cattle screening	Positive result n (%)	No. of milk samples taken	Positive result n (%)
A	25	13 (52)	23	15 (65.2)
B	25	17 (68)	32	23 (71.8)
C	25	18 (72)	26	22 (84.6)
D	25	12 (48)	22	14 (58.3)
Total	100	60 (60)	103	74 (71.8)
	P=0.365		P=0.344	

n (%)*: Number (percentage) of CMT-positive dairy cattle, and number (percentage) of positive milk samples (in the result of bacteriological analysis)*

Antibiotic Susceptibility Testing

The susceptibilities to ampicillin (AM, 10 µg), amoxicillin/clavulanic acid (AMC, 30 µg), cefotaxime (CTX, 30 µg), ciprofloxacin (CIP, 10 µg), erythromycin (E, 15 µg), gentamicin (CN, 10 µg), penicillin (P, 10 µg), tetracycline (TE, 30 µg) and trimethoprim/sulfamethoxazole (SXT, 25µg) (Bioanalyse, Türkiye) of the isolates were determined by the disk diffusion method (Bauer et al., 1966). *E. coli* ATCC 25922 was used as the standard control strain. The results were interpreted according to the breakpoints specified in the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2025) guidelines.

Statistical Analysis

The SCM prevalence was calculated according to CMT results in milk samples collected within the scope of the study. Prevalence data were represented by n (%). The chi-square test was used to analyze the statistical significance of differences in mastitis diagnosis according to the farms where milk samples were collected for CMT and the bacteriological analysis and the significance level was determined as $P < 0.05$. Statistical analyses were performed with the SPSS 30.01 package program.

A total of 75 isolates (2 isolates from one milk sample) were obtained (Table 2). As a result of phenotypic tests, 61 and 14 of the 75 isolates were defined as Gram-positive and Gram-negative bacteria. Six isolates were positive in the motility test. In addition, 17, 58, and 4 of the isolates were coagulase-positive, catalase-positive, and oxidase-positive, respectively. In the MALDI-TOF MS analysis, two and 66 of 75 isolates were identified at the genus level (*Psychrobacter* sp.) and the species level, respectively, but the remaining 7 isolates were not identified at the genus/species level. 16S rRNA gene sequencing was performed to identify 9 isolates (including two isolates identified at the genus level by MALDI-TOF MS). Five and 4 isolates were identified at the genus level (3 *Staphylococcus* sp. and 2 *Acinetobacter* sp.) and the species level (2 *Psychrobacter pulmonis*, 1 *Staphylococcus borealis* and 1 *Streptococcus pluranimalium*), respectively. The data of the 16S rRNA gene sequence of 9 isolates were deposited in GenBank under accession numbers from PQ870726 to PQ870734.

The most frequently identified genera at the genus level within 75 isolates were *Staphylococcus* (42/75),

Trueperella (8/75), *Escherichia* (5/75), and *Aerococcus* (5/75). The most identified *Staphylococcus* species at the species level were *S. aureus* (n=17), *S. haemolyticus* (n=13), *S. epidermidis* (n=4) and *S. chromogenes* (n=4), while the other most frequently identified species were *Trueperella pyogenes* (n=8), *Escherichia coli* (n=5), and *Aerococcus viridans* (n=5). In addition, two isolates recovered from one milk sample were defined as *T. pyogenes* and *S. aureus*. The distribution of the bacteria is presented in Table 2.

Table 2. The distribution of the recovered bacteria from milk samples

Genus	Species		No. of the isolates (n)	Percentage (%)	GenBank Accession Number
Staphylococcus	*S. aureus*		17	23	-
	CNS	*S. haemolyticus*	13	17.3	-
		S. chromogenes	4	5.3	-
		S. epidermidis	4	5.3	-
		S. borealis	1	1.3	PQ870733
		Staphylococcus sp.	3	4	PQ870727 PQ870730 PQ870731
Trueperella	*T. pyogenes*		8	10.7	-
Escherichia	*E. coli*		5	6.7	-
Aerococcus	*A. viridans*		5	6.7	-
Acinetobacter	*A. baumannii*		1	1.3	-
	A. johnsonii		1	1.3	-
	Acinetobacter sp.		2	2.7	PQ870728 PQ870732
Streptococcus	*S. agalactiae*		1	1.3	-
	S. uberis		1	1.3	-
	S. pluranimalium		1	1.3	PQ870726
Psychrobacter	*P. pulmonis*		2	2.7	PQ870729 PQ870734
Corynebacterium	*C. pseudotuberculosis*		1	1.3	
Arcanobacterium	*A. pluranimalium*		1	1.3	
Lactococcus	*L. lactis*		1	1.3	
Pasteurella	*P. multocida*		1	1.3	
Pseudomonas	*P. aeruginosa*		1	1.3	
Klebsiella	*K. oxytoca*		1	1.3	

***n**: No. of the isolates, **%**: Percentage of the isolates, **CNS:** Coagulase-negative Staphylococcus.*

The Results of the Antibiotic Susceptibility Testing

In the test, 48%, 32%, 30.6%, 26.7%, 22.7%, 17.6%, 14.7%, 9.3%, and 5.3% of the isolates were found to be resistant to CN, TE, P, AM, SXT, E, CIP, CTX, and AMC, respectively. The antibiotic resistance in 42 *Staphylococcus* spp. isolates was mostly detected to P (45.2%), CN (38.1%), and AM (35.7%). Of the 17 *S. aureus* isolates, 70.6%, 64.7%, 17.6%, 17.6%, 11.8%, 5.9%, and 5.9% were resistant to TE, CN, P, AM, E, AMC, and SXT, respectively. 64%, 48%, 24%, 20%, 16%, and 8% of 25 coagulase-negative *Staphylococcus* (CNS) isolates were found to be resistant to P, AM, SXT, CN, CTX, and E, respectively. The CN, SXT, and TE resistance rate of 8 *T. pyogenes* isolates was 62.5%. While the highest antibiotic resistance in 5 *E. coli* isolates was observed to CN (60%), AM (40%), and AMC (40%), the resistance in 5 *A. viridans* isolates was determined to CIP (100%) and CN (80%). In addition, *T. pyogenes* and *S. aureus* isolates recovered from one udder lobe were detected to be resistant to CN but susceptible to AM, AMC, CIP, CTX, and P. The detailed antibiotic susceptibility test results belonging to all isolates (n=75) are given in Table 3.

Table 3. The results of antibiotic susceptibility testing of the isolates obtained from milk samples

Isolates	Antibiotic																		
	AM		AMC		CIP		CN		CTX		E		P		SXT			TE	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	I	S	R	S
S. aureus	3 (17.6)*	14 (82.4)	1 (5.9)	16 (94.1)	-	17 (100)	11 (64.7)	6 (35.3)	-	17 (100)	2 (11.8)	15 (88.2)	3 (17.6)	14 (82.4)	1 (5.9)	3 (17.6)	13 (76.5)	12 (70.6)	5 (29.4)
CNS	12 (48)	13 (52)	-	25 (100)	-	25 (100)	5 (20)	20 (80)	4 (16)	21 (84)	2 (8)	23 (92)	16 (64)	9 (36)	6 (24)	2 (8)	17 (68)	-	25 (100)
T. pyogenes	-	8 (100)	-	8 (100)	2 (25)	6 (75)	5 (62.5)	3 (37.5)	-	8 (100)	4 (50)	4 (50)	-	8 (100)	5 (62.5)	-	3 (37.5)	5 (62.5)	3 (37.5)
E. coli	2 (40)	3 (60)	2 (40)	3 (60)	1 (20)	4 (80)	3 (60)	2 (40)	-	5 (100)	ND	ND	ND	ND	1 (20)	-	4 (80)	1 (20)	4 (80)
A. viridans	-	5 (100)	-	5 (100)	5 (100)	-	4 (80)	1 (20)	-	5 (100)	-	5 (100)	-	5 (100)	-	-	5 (100)	-	5 (100)
***Acinetobacter* spp.**	-	4 (100)	-	4 (100)	1 (25)	3 (75)	2 (50)	2 (50)	1 (25)	3 (75)	2 (50)	2 (50)	ND	ND	-	-	4 (100)	-	4 (100)
***Streptococcus* spp.**	1 (33.3)	2 (66.7)	-	3 (100)	-	3 (100)	3 (100)	-	1 (33.3)	2 (66.7)	1 (33.3)	2 (66.7)	-	3 (100)	1 (33.3)	1 (33.3)	1 (33.4)	1 (33.3)	2 (66.7)
P. pulmonis	-	2 (100)	-	2 (100)	-	2 (100)	1 (50)	1 (50)	-	2 (100)	1 (50)	1 (50)	ND	ND	1 (50)	-	1 (50)	1 (50)	1 (50)
C. pseudotuberculosis	-	1 (100)	-	1 (100)	-	1 (100)	1 (100)	-	-	1 (100)	-	1 (100)	-	1 (100)	-	-	1 (100)	-	1 (100)
A. pluranimalium	-	1 (100)	-	1 (100)	-	1 (100)	1 (100)	-	-	1 (100)	-	1 (100)	-	1 (100)	-	-	1 (100)	-	1 (100)
L. lactis	-	1 (100)	-	1 (100)	1 (100)	-	-	1 (100)	-	1 (100)	-	1 (100)	-	1 (100)	1 (100)	-	-	1 (100)	-
P. multocida	-	1 (100)	-	1 (100)	-	1 (100)	-	1 (100)	-	1 (100)	-	1 (100)	-	1 (100)	-	-	1 (100)	1 (100)	-
P. aeruginosa	1 (100)	-	1 (100)	-	1 (100)	-	-	1 (100)	1 (100)	-	ND	ND	ND	ND	1 (100)	-	-	1 (100)	-
K. oxytoca	1 (100)	-	-	1 (100)	-	1 (100)	-	1 (100)	-	1 (100)	ND	ND	ND	ND	-	-	1 (100)	1 (100)	-
Total (n=75)	**20 (26.7)**	**55 (73.3)**	**4 (5.3)**	**71 (94.7)**	**11 (14.7)**	**64 (85.3)**	**36 (48)**	**39 (52)**	**7 (9.3)**	**68 (90.7)**	**12 (17.6)**	**56 (82.4)**	**19 (30.6)**	**43 (69.4)**	**17 (22.7)**	**6 (8)**	**52 (69.3)**	**24 (32)**	**51 (68)**

n (%):* *No. of the isolates (%),* ***CNS:* *Coagulase-negative Staphylococcus,* ***ND:*** *Not Determined,* ***R:*** *Resistant,* ***I:*** *Intermediate,* ***S:*** *Susceptible,* ***AM:*** *Ampicillin,* ***AMC:*** *Amoxicillin/clavulanic acid,* ***CIP:*** *Ciprofloxacin,* ***CN:*** *Gentamicin,* ***CTX:*** *Cefotaxime,* ***E:*** *Erythromycin,* ***P:*** *Penicillin,* ***SXT:*** *Trimethoprim/sulfamethoxazole,* ***TE:*** *Tetracycline.*

Discussion and Conclusion

Mastitis, one of the leading diseases affecting milk capacity in dairy cattle, causes significant economic losses in dairy cattle farms in Türkiye and around the world (Tel et al., 2009; Tommasoni et al., 2023). CMT is an inexpensive, practical, and reliable test used to detect SCM cases in dairy cattle. The bacteriological analysis is performed to detect and identify bacteria that lead to SCM. As a result of the analysis, many bacteria, primarily *Staphylococcus* spp. are obtained in SCM cases (Tel et al., 2009). In the current study, 75 isolates were obtained as a result of bacteriological analysis of the milk samples from dairy cattle detected SCM after screening with CMT, and the most frequently identified species were *S. aureus* (23%), *S. haemolyticus* (17.3%), and *T. pyogenes* (10.7%). In the studies conducted in Türkiye, Tel et al. (2009) detected the most *S. aureus* (32.5%), CNS (27.5%), and *Streptococcus* spp. (8.9%) in the result of bacteriological analysis of 332 milk samples from cattle with SCM. Buyukcangaz et al. (2012) reported that the highest rates of *S. aureus* (31.45%), CNS (23.88%), and *Streptococcus* spp. (26.36%) were isolated and identified from 480 milk samples. Alkan et al. (2014) defined frequently *S. aureus* (20/34) and *Bacillus* spp. (7/34) in 109 CMT-positive/negative milk samples. Ozavcı et al. (2017) noted that it was mostly identified *Aeromonas hydrophila* (17.24%), *Corynebacterium* spp. (13.79%), *Lactobacillus* spp. (12.06%), *E. coli* (10.34%), *Shigella* sp. (10.34%), CNS (10.34%), and *S. aureus* (4.59%) from 238 milk samples. Kenar et al. (2019) obtained *E. coli* (13/61), *S. epidermidis* (8/61), *S. haemolyticus* (5/61), and *S. aureus* (4/61) as the most dominant species from 61 milk samples. Kahya Demirbilek (2020) detected the most frequent *Streptococcus* spp. (28.5%), *S. aureus* (23.1%), CNS (11.5%), and *E. coli* (11.5%) from 394 milk samples. Kurt and Eski (2021) analyzed 103 milk samples and determined that *E. coli* (19.9%), *S. aureus* (13.7%), and *Mycoplasma bovis* (8.2%) were the dominant species. Delikanlı Kıyak et al. (2024) reported that it was identified *E. coli* (26.10%), *S. aureus* (21.29%), *Str. agalactiae* (20.08%), and *S. epidermidis* (12.05%) in the analysis of the milk samples. In the studies conducted worldwide, Barreiro et al. (2010) noted that it was obtained 33 isolates from milk samples from cattle with SCM, and 42.4%, 30.3%, and 27.3% of them were identified as *S. aureus*, *Str. agalactiae* and CNS, respectively. Persson et al. (2011) reported that the most common species identified in 583 milk samples were *S. aureus* (19%), CNS (16%), and *Str. dysgalactiae* (9%). Dieser et al. (2014) detected mostly CNS (52.1%) and *S. aureus* (21.3%) in 1201 milk samples. Abed et al. (2021) defined the most frequently *E. coli* (49.8%), *S. aureus* (44.9%), *Streptococcus* spp. (44.1%) and non-aureus staphylococci (37.1%) in 488 milk samples. Al-Harbi et al. (2021) announced that it was identified commonly non-aureus staphylococci (10%), *Str. uberis* (7%), and *S. aureus* (5%) in milk samples. Chung et al. (2021) determined by a majority of CNS (44.7%), *Bacillus* spp. (30.3%), and *S. aureus* (20.45%) in 132 milk samples. Huma et al. (2022) identified the most *S. aureus* (34.45%), *E. coli* (21.36%), and CNS (13.54%) in 235 milk samples. Michira et al. (2023) declared that it was defined frequently CNS (40.1%), *S. aureus* (15.8%), and *Micrococcus* spp. (10.4%) in milk samples. Cao et al. (2023) noted that the most common isolates from 208 milk samples were *K. pneumoniae* (15.35%), *E. coli* (13.70%), and *P. aeruginosa* (12.33%). Rifatbegović et al. (2024) obtained the highest *S. aureus* (17.1%), CNS (17.1%), *Str. uberis* (12.2%), *Streptococcus* spp. (12.2%), and *T. pyogenes* (9.8%) from 111 milk samples. In the majority of the studies mentioned above, *S. aureus* and non-aureus staphylococci were the most commonly identified bacteria in dairy cattle with SCM, which is consistent with our study's results. It is thought that minor differences in the distribution of the most frequently identified species may be due to geography, nutrition, farm management, hygienic conditions, milking practices, and isolation/identification methods (Kasa et al., 2020).

Antibiotic treatment is an important therapy for the prevention and control of mastitis cases. On the other hand, the extensive empirical use of antibiotics is known to be the primary cause of AMR (Abed et al., 2021). There are many studies on determining the antibiotics to be used in the treatment of SCM cases, and the antibiotic resistance profiles show differences in these studies. The resistance rates of *S. aureus* isolates recovered from dairy cattle with SCM in Türkiye to AM, AMC, CIP, CN, CTX, E, P, SXT, and TE ranged between 10.5%-77.3%, 10%-66.5%, 0%-50%, 0%-59.5%, 2.9%-25%, 0%-63%, 0%-75.8%, 3.6%-12.6%, and 17.9%-23.3%, respectively (Tel et al., 2009; Ozavcı et al., 2017; Gulmez Saglam et al., 2018; Kenar et al., 2019; Kahya Demirbilek, 2020; Gokdag and Ciftci, 2021; Tavsanli and Cibik, 2022). It is observed that the resistance rates of *S. aureus* isolates worldwide to AM, AMC, CIP, CN, CTX, E, P, SXT, and TE varied between 45.7%-96%, 0%-78%, 0%-16%, 0%-32.1%, 0%-46%, 0%-44.6%, 4%-92.7%, 0%-26%, and 0%-18.6%, respectively (Persson et al., 2011; Xu et al., 2015; Ren et al., 2020; Abed et al., 2021; Al-Harbi et al., 2021; Chung et al., 2021; Michira et al., 2023; Silva et al., 2023; Rodríguez et al., 2023; Haq et al., 2024). In our study, it is detected that 70.6%, 64.7%, 17.6%, 17.6%, 11.8%, 5.9%, and 5.9% of the 17 *S. aureus* isolates were resistant to TE, CN, P, AM, E, AMC, and SXT, respectively (Table 3). TE and CN resistance rates in *S. aureus* isolates are higher than the antibiotic resistance rates mentioned above.

Of the CNS or non-aureus staphylococci isolates obtained from dairy cattle with SCM in Türkiye, the

resistance rates against AM, AMC, CIP, CN, CTX, E, P, SXT, and TE were found to vary between 8.5%-58%, 8.6%-89%, 0%-56%, 0%-66.2%, 2.9%-47.4%, 5.6%-46.8%, 11.4%-55.3%, 2.8%-6.4%, and 0%-40.4%, respectively (Tel et al., 2009; Buyukcangaz et al., 2012; Ozavcı et al., 2017; Gulmez Saglam et al., 2018; Kenar et al., 2019; Kahya Demirbilek, 2020; Gokdag and Ciftci, 2021). It was determined that the resistance rates of CNS or non-aureus staphylococci isolates worldwide to AM, AMC, CIP, CN, CTX, E, P, SXT, and TE ranged between 27%-90%, 0%-76%, 0%-12%, 0%-18%, 0%-42%, 2%-49%, 12%-86.8%, 0%-16.1%, and 1%-39.5%, respectively (Botrel et al., 2010; Persson et al., 2011; Xu et al., 2015; Phophi et al., 2019; Abed et al., 2021; Al-Harbi et al., 2021; Chung et al., 2021; Michira et al., 2023; Rodríguez et al., 2023). In the present study, it determined that 64%, 48%, 24%, 20%, 16%, and 8% of 25 CNS isolates were resistant to P, AM, SXT, CN, CTX, and E, respectively (Table 3). The resistance rate of SXT in CNS isolates was detected to be higher than those mentioned above. It is thought that these differences in antibiotic resistance profiles of both *S. aureus* and CNS isolates in our study may be related to geographic location, climate, population characteristics, nutrition, hygienic conditions, study design, the number of isolates, etc. (Haq et al., 2024).

In conclusion, the subclinical mastitis rate in dairy cattle in Kayseri province of Türkiye, for the CMT results was detected to be 25.75%, and 71.8% positivity (74/103) was detected as a result of bacteriological analysis. The most commonly identified genus and species among the recovered 75 isolates were *Staphylococcus* (56%) and *S. aureus* (22.7%), respectively. The gentamicin (48%) and tetracycline (32%) resistance were detected at a high rate in the 75 isolates analyzed according to the antibiotic susceptibility testing results. Due to antibiotic resistance, it is thought that it may be appropriate to take various precautions to prevent the emergence/spread of the resistance in dairy cattle farms in the region. In addition, the present study showed that there may be a need for dairy farms in this region to develop useful strategies for the treatment/control of SCM, according to the recovered bacteria from SCM cases and antibiotic resistance profiles.

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Competing Interest

The authors declare that they have no conflict of interest.

Ethical Statement

Ethics committee approval is not required within the scope of this study.

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