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RESEARCH ARTICLE

Detection of Bacterial Isolation and Antimicrobial Resistance Profiles in Goat Mastitis

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Abstract

Mastitis is one of the most common infections worldwide. This infection poses risks to animal and public health. Therefore, determination of mastitis pathogens is important for the prevention of the infection. Generally, the combination of antimicrobials is an effective, reliable and common treatment approach. In this study, it was aimed to determine the bacterial mastitis pathogens in goat's milk and antimicrobial resistance profiles of these pathogens. A total of 190 goat milk samples were examined with standard microbiological analysis in the period of 2018-2019. Bacterial mastitis pathogens were obtained in 33.2% of all samples. Totally, 79 different bacterial agents were isolated due to multiple-bacterial isolation. Major genus was detected as *Staphylococcus* spp. (66%): coagulase-negative *Staphylococcus* (CNS) 44% and coagulase-positive *Staphylococcus* (CPS) 22%. This group was followed by respectively *Bacillus* spp. (17.6%), catalase-negative cocci (PNC) (14%), *Mannheimia* spp. (1.2%) and *Micrococcus* spp. (1.2%). The most prevalent species were identified *Staphylococcus caprae* (27%), *Staphylococcus aureus* and *Staphylococcus chromogenes* (13%), *Aerococcus viridans* and *Bacillus cereus* (7.6%). Considering the antimicrobial resistance test, tetracycline has the highest resistance rate (31%) among the tested antimicrobials. A total of 4 multi-drug resistant isolates were found: an *Enterococcus faecalis* and three *Streptococcus uberis* isolates. The highest resistance rate (35.7%) was observed for penicillin in all *Bacillus* spp. isolates.

Keywords: *Coagulase-negative Staphylococcus, Goat milk, Mastitis, Staphylococcus caprae*

Keçi Mastitisinde Bakteriyel İzolasyon ve Antimikrobiyal Direnç Profillerinin Tespiti

Öz

Mastitis, dünya çapında en yaygın enfeksiyonlardan biridir. Bu enfeksiyon, hayvan ve halk sağlığı açısından risk oluşturmaktadır. Bu nedenle, mastitis patojenlerinin belirlenmesi enfeksiyonun önlenmesi açısından önemlidir. Antimikrobiyal tedavi, bakteriyel mastitis tedavisinde en güvenilir ve en yaygın olanıdır. Genel olarak birden fazla antimikrobiyalin kombinasyonu, etkili bir tedavi yaklaşımıdır. Bu çalışmada, keçi sütündeki bakteriyel mastitis patojenlerinin saptanması ve bu patojenlerin antimikrobiyal direnç profillerinin belirlenmesi amaçlanmıştır. 2018-2019 yılları arasında toplam 190 keçi sütü örneği standart mikrobiyolojik analizler ile incelendi. Tüm numunelerinin %33.2'sinde bakteriyel mastitis patojenleri tespit edildi. Çoklu bakteriyel izolasyonu nedeniyle toplam 79 farklı bakteri izole edildi. Major bakteri cinsi, %44 koagülaz-negatif Staphylococcus (CNS) ve %22 koagülaz-pozitif *Staphylococcus* (CPS) olmak üzere *Staphylococcus* spp. (%66) olarak belirlendi. *Staphylococcus* cinsini takiben sırasıyla *Bacillus* spp. (%17.6), katalaz-negatif koklar (PNC) (%14), *Mannheimia* spp. (%1.2) ve *Micrococcus* spp. (%1.2) izole edildi. En yüksek prevelansa sahip türler *Staphylococcus caprae* (%27), *Staphylococcus aureus* ve *Staphylococcus chromogenes* (%13), *Aerococcus viridans* ve *Bacillus cereus* (%7.6) olarak tanımlandı. Antimikrobiyal direnç testi dikkate alındığında, test edilen antimikrobiyaller arasında en yüksek direnç tetrasiklinde (%31) tespit edildi. Bir *Enterococcus faecalis* ve üç *Streptococcus uberis* olmak üzere toplam 4 adet çoklu-ilaç dirençli izolat bulundu. Tüm *Bacillus* spp. izolatlarında en yüksek direnç oranı (%35.7) penisilin için gözlendi.

Anahtar sözcükler: *Keçi sütü, Koagülaz-negatif Staphylococcus, Mastitis, Staphylococcus caprae*

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Introduction

Goat milk is a highly nutritious milk containing proteins, minerals and vitamins. The production of goat milk is approximately 19 million tons worldwide [1]. However, milk quality is negatively affected by many factors such as mastitis. Mastitis (intramammary infections, IMI), is one of the most common infections worldwide. IMI affects animals and decreases productivity, quality in milk production industry. This infection also causes economic problems due to treatment and yield decline. Therefore, determination of IMI pathogens is important to control and prevent the infection.

A wide range of, approximately 140, microorganisms are the cause of IMI [2]. The major bacterial pathogens of IMI are coagulase-negative *Staphylococccus* spp., *Staphylococcus aureus, Streptococcus uberis, Streptococcus dysgalactiae, Corynebacterium* spp., *Escherichia coli, Klebsiella* spp.[3]. *Staphylococcus* food poisoning is defined as one of the common foodborne diseases around worldwide. Especially, *S. aureus* IMI is a risk to public health via secretion of enterotoxins [4]. Gram-positive catalase-negative cocci (PNC) have also been responsible for goat IMI. The most common PNC species isolated in IMI are *S. uberis, S. dysgalactiae, Streptococcus agalactiae, Enterococcus faecalis* and *Aerococcus viridans*. Frequently, they have been defined as environmental pathogens in IMI and they are transmitted to ruminants via farm equipment [5]. One of the environmental mastitis pathogens is *Bacillus* spp. has also been identified in clinical and subclinical IMI in previous studies. It poses a risk to producers and consumers of milk in terms of causing zoonotic disease [4,6]. *Bacillus cereus, Bacillus subtilis, Bacillus licheniformis, Bacillus thuringiensis* and *Bacillus circulans* are observed in goat IMI [7]. *Mannheimia* species are frequently observed in pneumonia, they are rarely detected in IMI cases. *M. haemolytica*, *M. glucosida* and *M. ruminalis* was previously investigated in IMI cases as studied by Poulsen et al.[8]. Especially in small ruminants, they have negatively affected animal welfare, productivity, production and quality of meat/milk. Even Omaleki et al.[9] have reported that *Mannheimia* species can be more important than *S. aureus* as a cause of IMI. Considering all these bacterial pathogens, antimicrobial treatment is commonly used to control bacterial IMI in small ruminants as a reliable method. The efficiency of treatment is dependent on the bacterial pathogen, susceptibility of antimicrobial, breed, clinical and environmental conditions. Generally, a combination of more than one antimicrobial is effective to control IMI than using of a single antimicrobial [6,10].

The present study was aimed to determine bacterial mastitis pathogens in goat's milk and antimicrobial resitance profiles of these pathogens. For this purpose, a total of 190 goat milk samples were examined by standard bacteriological analysis and tested for antimicrobials.

Material and Methods

Collection of Samples

The goat milk samples were obtained from a dairy goat farm in the period 2018-2019 located in Ankara, Turkey. A physical examination and California Mastitis Test (CMT) was used to detect mastitis. The samples were taken from positives in the CMT, according to aseptic techniques using sterile gloves, overall and tubes after the cleaning of teats with 70% ethyl alcohol [2]. After drying of alcohol, the first streams were discarded. Approximately 15 mL of individual milk samples were collected from each teat to sterile tube horizontally. In total, 190 milk samples were collected from 190 goats and immediately transported at cold chain. The samples were stored at 4°C until bacteriological analysis [1].

Bacterial Isolation and Identification

Mastitis causing pathogens were identified using standard bacteriological methods. For each milk sample, a loopful sample [~10 µL] was plated on 5% sheep blood agar plates and the plates were incubated at 37°C 24-72 s in aerobic conditions [1]. After incubation, bacterial colonies were separated according to morphological features such as pigmentation, colony form, hemolytic characteristics and purified on new plates. All isolates were examined by Gram staining, catalase, oxidase and oxidation-fermentation (OF) tests. The bacteria were identified using the methods described by Markey et al.[11]. *Staphylococcus* spp. isolates were identified to species based on coagulase tube test, urease production, DNase activity, mannitol fermentation test, polymixin-B test (300 U/disc), novobiocin test (5 µg/disc). Gram-positive cocci in clusters, catalase-positive, coagulase-negative, oxidative, bacitracin test (0.04 U/disc) susceptible bacteria were classified in *Micrococcus* spp. Gram-positive, catalase-negative cocci (PNC), were identified at genus level based on cultures of Edwards agar, MacConkey agar. For PNC, species-level identification was performed by 16S *rRNA* gene sequence analysis. *Enterococcus* spp. isolates were separated by esculin hydrolysis on Edwards agar and growth on MacConkey agar from *Streptococcus* spp. *Bacillus* spp. isolates were identified based on sporulation, nitrate reduction, motility test, hemolysis, MacConkey agar, urease production, Voges-Proskauer, citrate, glucose, penicillin susceptibility. *Mannheimia* spp. isolate was identified based on indole production, nitrate reduction, hemolysis, motility test, growth on MacConkey agar, urease production [7,8,11]. The conventional identification results were confirmed with the BD Phoenix M50 Instrument (BD, USA). Specific identification kits were used for Gram positive and Gram negative taxa in this automated micro-biology system.

16S rRNA Gene Sequence Analysis

DNA extraction was performed with GeneJET Genomic DNA Purification kit, Gram Positive Bacterial DNA extraction protocol (Thermo Fisher Scientific, USA). PCR amplification

of the 16S rRNA gene was performed using a 25 µL PCR reaction, which contained 12.2 µL PCR-grade water, 2.5 µL of 10xbuffer, 0.5 µL 10 mM dNTPs, 2.5 µL $MgCl_2$, 1 µL of each 10 mM primer, 0.3 µL Taq (2U/ µL) and 5 µL template DNA (Thermo Fisher Scientific, USA). For amplification and sequencing, 27F and 1492R universal primers were used [12]. The PCR conditions were as follows: pre-denaturation at 94°C for 4 min, then 35 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 1 min and final extension at 72°C for 6 min. The PCR products were purified with Exosap-IT (Thermo Fisher Scientific, USA) by incubation at 37°C for 45 min and 80°C for 15 min. BigDye Terminator v3.1 Cycle Sequencing Kit was used for sequence analysis (Thermo Fisher Scientific, USA). The cycle PCR products were purified with Sephadex gel filtration (Oxoid, UK). DNA samples were sequenced on ABI 3500 genetic analyzer system (Applied Biosystems, USA) and were aligned by CLC Main Workbench v.8.0.1 sequence analysis program (Qiagen, USA). DNA sequences were compared by National Centre for Biotechnology Information (NCBI, USA) BLASTN server for identification at the species level (≥99% sequence similarity).

Antimicrobial Resistance Test

Kirby-Bauer Disc Diffusion method was used following the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2008) [13]. Ampicillin (AMP, 10 µg/disc), cefoxitin (FOX, 30 µg/disc), ciprofloxacin (CIP, 5 µg/disc), clindamycin (DA, 2 µg/disc), cefotaxime (CTX, 30 µg/disc), ceftriaxone (CRO, 30 µg/disc), doxycycline (DO, 30 µg/disc), erythromycin (E, 15 µg/disc), gentamycin (CN, 10 µg/disc), kanamycin (K, 30 µg/disc), ofloxacin (OFX, 5 µg/disc), penicillin-G (P, 10 µg/disc), rifampin (RD, 5 µg/disc), tetracycline (TE, 30 µg/disc), trimethoprim (W, 5 µg/disc) and vancomycin (VA, 30 µg/disc) antimicrobial agents were tested. Ten antimicrobials were tested for *Staphylococcus* spp. isolates: FOX, CIP, DA, E, CN, K, P, TE, W and VA. The methicillin-resistant *Staphylococcus* (MRS) was determined with cefoxitin because cefoxitin is more sensitive detection of mecA-positive isolates [14]. Nine antimicrobials were tested for *Streptococcus* spp. isolates: AMP, DA, CTX, CRO, E, OFX, P, TE and VA. Seven antimicrobials were tested for *Enterococcus* spp. isolates: AMP, CIP, DO, E, RD, TE and VA. After incubation at 35°C±2°C 16-24 s, zone of inhibition was measured (mm). For *Bacillus* spp. isolates, MICs of seven antimicrobial agents (CIP, DA, E, CN, P, TE and VA) were determined by the Broth Microdilution method. The evaluation of results were performed according to the CLSI, 2008. Antimicrobial resistance (AMR) test of *A. viridans, Micrococcus luteus* and *Mannheimia ruminalis* isolates were not performed because of which organisms have excluded from CLSI protocol. Multi-drug resistant (MDR) isolates were detected by resistance to three or more classes of antimicrobials [15].

Results

A total of 190 milk samples were examined with standard microbiological analysis. The conventional identification results were 100% compatible with the results of BD Phoenix M50 automated microbiology system. Sixty three milk samples (33.2%) were found positive for bacterial IMI agents. Totally, 79 different bacterial agents were isolated due to the isolation of more than one bacteria in 15 samples. The combination of different two species were obtained at 14 samples, while the combination of different three species was obtained in one sample *(Table 1)*. The isolates were CNS (44%), CPS (22%), *Bacillus* spp. (17.6%), PNC (14%), *Mannheimia* spp. (1.2%) and *Micrococcus* spp. (1.2%). As a result of 16S rRNA sequence analysis performed for PNC, 6 *A. viridans* and 3 *S. uberis* were determined. The most common species identified were *Staphylococcus caprae* (27%, 21/79), *S. aureus* (13%, 10/79) and *S. chromogenes* (13%, 10/79). The other isolates were identified as belonging to *A. viridans* and *B. cereus* (7.6%, 6/79); *B. pumilis, S. intermedius* and *S. kloosii (*5%, 4/79); *S. hyicus* and *S. uberis* (3.8%, 3/79); *B. licheniformis* and *E. faecalis* 2.6%; *B. thuringiensis, B. circulans, M. luteus,* and *M .ruminalis* (1.2%, 1/79) *(Table 2)*.

Antimicrobial resistance test of the *Staphylococcus* isolates

Table 1. *Species combination and number of positive samples*

Species Combination		Number of Positive Samples
One species	*S. caprae*	11
	S. aureus	9
	B. cereus	6
	A.viridans	5
	S. uberis	3
	E. faecalis	2
	S. intermedius	2
	S. hyicus	2
	B. circulans	1
	B. licheniformis	1
	B. pumilis	1
	B. thuringiensis	1
	M. luteus	1
	M. ruminalis	1
	S. chromogenes	1
	S. kloosii	1
Two species	*S. caprae, S. chromogenes*	4
	S. caprae, S. kloosii	3
	S. chromogenes, B. pumilis	2
	S. chromogenes, S. intermedius	2
	S. aureus, S. caprae	1
	S. caprae, B. pumilis	1
	S. hyicus, A. viridans	1
Three species	*S. caprae, S. chromogenes, B. licheniformis*	1
Total		63 (33.2%)

Table 2. *Prevalence and etiology of bacterial IMI in goat milk*

Group (%)	Species	Number of Isolates (%)
CNS[a] (44)	*S. caprae* *S. chromogenes* *S. kloosii*	21 (27) 10 (13) 4 (5)
CPS[b] (22)	*S. aureus* *S. intermedius* *S. hyicus*	10 (13) 4 (5) 3 (3.8)
Bacillus (17.6)	*B. cereus* *B. pumilis* *B. licheniformis* *B. circulans* *B. thuringiensis*	6 (7.6) 4 (5) 2 (2.6) 1 (1.2) 1 (1.2)
PNC[c] (14)	*A. viridans* *S. uberis* *E. faecalis*	6 (7.6) 3 (3.8) 2 (2.6)
Micrococci (1.2)	*M. luteus*	1 (1.2)
Mannheimia (1.2)	*M. ruminalis*	1 (1.2)
Total		79 (100)

[a] *coagulase-negative Staphylococcus,* [b] *coagulase-positive Staphylococcus,* [c] *catalase-negative cocci*

indicated that they were susceptible to ciprofloxacin, clindamycin, cefoxitin, erythromycin, gentamycin, trimethoprim, and vancomycin *(Table 3)*. Among the *Staphylococcus* species, tetracycline has the highest resistance rate (32.6%, 17/52) among the tested antimicrobials. There was no detection of MRS and MDR among *Staphylococcus* isolates. The resistance to maximum two antimicrobials (penicillin and tetracycline) was detected for an *S. caprae* and an *S. chromogenes*. The *S. hyicus* isolates were susceptible to all antimicrobials. All of the *Bacillus* isolates were found susceptible to clindamycin, erythromycin, gentamycin, tetracycline and vancomycin *(Table 3)*. Among the tested antimicrobials, Beta lactam antimicrobial such as penicillin has the highest resistance rate (35.7%, 5/14). There is no detection of MDR between *Bacillus* isolates. The resistance to maximum two antimicrobials (penicillin and ciprofloxacin) was detected for a *B. cereus*. AMR test of the *Enterococcus* isolates indicated that they were susceptible to ampicillin, ciprofloxacin, penicillin *(Table 3)*. Among the tested antimicrobials, tetracycline has the highest resistance rate (100%, 2/2). An *E. faecalis* MDR isolate was found with resistance to rifampin, tetracycline and vancomycin. The other *E. faecalis* isolate was found

Table 3. *The AMR test profiles of isolates*

Antimicrobials[a]	Species	Number of Isolates (%)	
		Susceptibles	AMR Profiles
CIP, CN, DA, E, FOX, K, P, TE, VA, W	*S. caprae* (n=21)	15 (71.4)	TE, 4 (19) K, 1 (4.8) TE-P, 1 (4.8)
	S. aureus (n=10)	7 (70)	TE, 3 (30)
	S. chromogenes (n=10)	2 (20)	TE, 6 (60) TE-P, 1 (10) DA, 1 (10)
	S. intermedius (n=4)	3 (75)	TE, 1 (25)
	S. kloosii (n=4)	2 (50)	TE, 1 (25) K, 1 (25)
	S. hyicus (n=3)	3 (100)	-
	B. cereus (n=6)	4 (66.6)	P, 1 (16.7) CIP-P, 1 (16.7)
CIP, CN, DA, E, P, TE, VA	*B. pumilis* (n=4)	4 (100)	-
	B. circulans (n=1)	-	P, 1 (100)
	B. licheniformis (n=2)	1 (50)	P, 1 (50)
	B. thuringiensis (n=1)	-	P, 1 (100)
AMP, CRO, CTX, DA, E, OFX, P, TE, VA	*S. uberis* (n=3)	-	E-TE-DA, 3 (100)
AMP, CIP DO, E, RD, TE, VA	*E. faecalis* (n=2)	-	TE-VA-RD, 1 (50) E-TE, 1 (50)

[a] ***AMP:*** *Ampicillin;* ***CIP;*** *ciprofloxacin;* ***CN:*** *gentamycin,* ***CRO:*** *ceftriaxone;* ***CTX:*** *cefotaxime;* ***DA:*** *clindamycin;* ***DO:*** *doxycycline;* ***E:*** *erythromycin;* ***FOX:*** *cefoxitin;* ***K:*** *kanamycin,* ***OFX:*** *ofloxacin;* ***P:*** *penicillin-G;* ***RD:*** *rifampin;* ***TE:*** *tetracycline;* ***VA:*** *vancomycin;* ***W:*** *trimethoprim*

Table 4. *The AMR test profiles of isolates from a single milk sample*

Species Combination	Number of Samples	Common Resistance Profile (%)
S. caprae, S. chromogenes	4	Tetracycline (26.6)
S. caprae, S. kloosii	3	Tetracycline and Penicillin (20)
S. chromogenes, B. pumilis	2	Tetracycline and Penicillin (13.3)
S. chromogenes, S. intermedius	2	Tetracycline and Clindamycin (13.3)
S. aureus, S. caprae	1	-
S. caprae, B. pumilis	1	-
S. hyicus, A. viridans	1	-
S. caprae, S. chromogenes, B. licheniformis	1	Tetracycline (13.3)

resistant to erythromycin and tetracycline. Considering the AMR test of *S. uberis* isolates, they were susceptible to ampicillin, cefotaxime, ceftriaxone, ofloxacin, penicillin and vancomycin. All three *S. uberis* isolates were found MDR with resistance to erythromycin, clindamycin and tetracycline. Different agents isolated from a single milk sample showed some common resistance in the AMR test. Tetracycline resistance was common in 86.5% of multiple isolations, penicillin resistance in 33.3% of multiple isolations, and clindamycin resistance in 13.3% *(Table 4)*.

Discussion

This study was carried out to detect prevalence, diversity and antimicrobial resistance of the bacterial IMI agents in goat milk. Bacteria isolation was performed in 33.2% of the samples. 66% of all isolates were identified as *Staphylococccus*. These rates are in consistent to reports by Ebrahimi et al.[16] and Omar et al.[4]. CNS were detected 44% and CPS were detected 22% among *Staphylococcus* isolates. Cremonesi et al.[17] had reported CNS are part of microbiota, they can be considered as minor-mastitis pathogen in IMI and have become predominant pathogens causing IMI [8]. This study has shown that CNS are major bacteria causing IMI in goats. *S. epidermidis* was reported as the most dominant CNS species in the goat mastitis study carried out by Danmallam and Pimenov [18]. Similarly, Ebrahimi et al.[16] and Ruiz et al.[19] had also reported that *S. epidermidis* was the dominant CNS. However, in this study, *S. caprae* was found to be the most common CNS with a prevalence of 27%. This situation was thought to be due to the survival capacity of *S. caprae* during lactation and dry period. There are several studies that support this finding and report *S. caprae* is dominant in subclinical and clinical cases [20,21]. Moreover, *S. caprae,* the human pathogen, is known to cause hospital infections such as endocarditis, meningitis, bone infections, peritonitis, pneumonia and urinary infections. This situation reveals the importance of goat milk pasteurization or IMI treatment for public health [8]. *S.aureus* was detected as the most common CPS with prevalence of 13%, which is an agent of all samples. It is in concordance with Ebrahimi et al.[16], Danmallam and Pimenov [18] and Ruiz et al.[19] who also found that *S. aureus* was the most common CPS. *S. aureus* IMI is a potential risk for public health due to the fact that it can cause food poisoning with enterotoxins and virulence factors. Therefore, the high prevalence of *S. aureus* is considerable. As a result of this study, the most prevalent second genus was *Bacillus* spp. *Bacillus* spp. has also been identified in clinical IMI and subclinical IMI in many previous studies [1,6]. *Bacillus* spp. are known as the environmental mastitis pathogens. They cause milk contamination by direct contact to teats or indirect contact with materials. However, they pose risk to producers and consumers of milk in terms of causing zoonotic diseases [4]. This study has shown that 17.6% of all bacterial isolates were *Bacillus* spp. The high prevalence can be probably related to low hygienic conditions. *Bacillus* isolates were identified as *B. cereus, B. pumilis, B. licheniformis, B. thuringiensis and B. circulans*. *B. cereus* was reported as a causative agent of food poisoning and gangrenous IMI in goats as mentioned by Aruwa et al.[7] and Mavangira et al.[22]. Besides, Mavangira et al.[22] has reported that *B. cereus* causes gastrointestinal and non-gastrointestinal infections in humans. After *Bacillus* spp., PNC was the fourth mostly isolated group in this study. They can cause clinical or subclinical IMI and have also been isolated in goat IMI by previous studies [2,6,8,23]. While *S. agalactiae* was determined to be major PNC in many other IMI studies, however it was not detected in this study [2]. However, Raemy et al.[5] stated that *S. uberis* was the dominant PNC in their IMI study. This finding is consistent with our rate. Finally, we isolated *M. luteus* and *M. ruminalis* with prevalence of 1%. These species are generally reported with low prevalence in IMI studies of goats. For example, 2.5% prevalence for *M. luteus* was reported by Danmallam et al.[18] *M. ruminalis* was reported with 1.2% prevalence by Omaleki et al.[9] in small ruminant IMI. *Mannheimia* species naturally exist in the upper respiratory system of small ruminants. They are also rarely detected in IMI cases. There are several studies on the investigation of the *Mannheimia* species such as *M. haemolytica*, *M. glucosida* and *M. ruminalis* in IMI cases [8,16]. Especially in small ruminants, they negatively affect animal welfare, productivity, production and quality of meat/milk [9].

IMI was considered the main economic problem in dairy goats [1]. Therefore, its treatment is important to prevent

loss of value and efficiency. Antimicrobial treatment is commonly used as an appropriate and reliable method to control bacterial IMI in small ruminants. Generally, a combination of more than one antimicrobial is effective to control IMI than just using a single antimicrobial [10,24]. The antimicrobial resistance test of the isolates showed that the highest resistance rate (31%) was seen in tetracycline. Ebrahimi et al.[16] reported was average 26.8% MRS; Bochev and Russenova [25] reported 20% MRS in *Staphylococcus* isolated from goat IMI. However, there is no detection of MRS and MDR among *Staphylococcus* isolates in the present study. A total of 4 MDR isolates were detected in PNC: an *E. faecalis* isolate resistant to rifampin, tetracycline, vancomycin and three *S. uberis* isolates resistant to erythromycin, clindamycin, tetracycline. AMR test of the *Bacillus* isolates indicated that they were susceptible to clindamycin, erythromycin, gentamycin, tetracycline and vancomycin. Penicillin has the highest resistance rate (35.7%). This rate showed that Beta lactams can be used successfully in the treatment of *Bacillus* in acute IMI, rather than other antimicrobials. There was no detection of MDR among *Bacillus* isolates.

In this study, *S.caprae S.chromogenes* and *S.aureus* were determined as the most prevalent mastitis agents in dairy goats. CNS species were found to have a higher prevalence than CPS species.

Unlike the most mastitis studies performed on goat milk, it was determined that the dominant CNS species was *S. caprae* instead of *S. epidermidis*. This is thought to be due to high persistence of *S. caprae*. CNS species are considered as minor pathogens in goats due to their limited pathogenicity. *S. aureus* is considered risky for public health and animal health due to its high pathogenicity. *S. aureus* prevalence was found to be low in this study. The isolation of environmental factors such as *Bacillus* spp. and *Streptococcus* spp., low hygienic conditions have shown that the prevalence of IMI agents is increased. AMR results showed that treatment with more than one antimicrobial combination would be a necessary and effective treatment for goat mastitis. It was found that a successful treatment is possible, given the multiple bacterial isolations and the high resistance of tetracycline and penicillin.

Availability of Data and Materials

The authors declare that data supporting the findings of this study are available upon request.

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This research received no grant from any funding agency/ sector.

Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

SSI, contributed to writing, reviewing and methodology of manuscript. AB and SS are responsible of sampling. ED and TO contributed draft preparation. MA contributed editing and study design. All authors have approved the final manuscript.

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