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Research Article

Detection of Antimicrobial Resistance in *Staphylococcus aureus* Isolated from Saanen Goats with Subclinical Mastitis

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A B S T R A C T

Background/Aim: The aim of the present study is to describe the distribution of viral antigen localization in nonnervous tissues was described in goats naturally infected with caprine arthritis encephalitis virus.

Materials and Methods: The milk samples were collected from one Saanen goats flock in the Western Turkey. A total of 280 half-udder milk samples (140 Saanen goats) were collected in a single sampling time in a flock during the early lactation period. The milk samples were incolated to 5 % sheep blood agar incubated at 37°C for 24-48 hours. After that, all isolates were identified as *S. aureus*, using biochemical tests and using API ID32 STAPH system (bioM´erieux®).

Results and Conclusion: In this study, bacterial isolation was made from 138 (49.3%) out of 280 milk samples taken from subclinical mastitic milk of Saanen goat. Bacterial isolates were identified as *S. aureus* 90 (32.1%). All *S. aureus* isolates (n=90) were subjected to antimicrobial susceptibility testing. *S. aureus* isolates were resistant to oxytetracycline 100%, ampicillin 91% and vancomycin 60%. *S. aureus* are more in number as causative agents in subclinical mastitis and novobiocin is the most effective antibiotic in Saanen subclinical mastitis.

Keywords: Saanen Goat, Subclinical Mastitis, S. aureus, Identification, Antimicrobial resistance.

Subklinik Mastitisli Saanen Keçilerinden İzole Edilen *Staphylococcus aureus* İzolatlarının Antimikrobiyel Dirençlerinin Belirlenmesi

ÖZET

Özbilgi/Amaç: Mastitis, bir ya da birden çok mikroorganizma tarafından oluşturulan meme bezinin yangısı ile karakterize bir hastalıktır. Süt içinde bulunan mikroorganizmaların varlığı genellikle mikrobiyel kültür yöntemi ile ortaya konabilmektedir. *Staphylococcus aureus*, keçilerde en sık teşhis edilen ve meme içi infeksiyonlara yol açan mikroorganizmadır. Bu çalışmanın amacı, Saanen keçilerinden izole edilen *S. aureus* etkenlerine karşı antimikrobiyel ajanların dirençliliğini tespit etmektir.

Materyal ve Metot: Süt örnekleri, Batı Türkiye bölgesinde bulunan bir Saanen keçisi çiftliğinden toplanmıştır. Erken laktasyon periyodunda bulunan her bir hayvanın iki meme lobundan olmak üzere 280 adet (140 Saanen keçisi) süt numunesi tek sefer örnekleme ile toplanmıştır. Toplanan süt numuneleri, % 5 koyun kanlı agara ekilmiş ve 37°C'de 24-48 saat inkube edilmiştir. Daha sonra, bütün izolatlar için biyokimyasal testler ve API ID32 STAPH sistemi (bioM´erieux®) kullanılmış ve izolatlar *S. aureus* olarak identifiye edilmiştir.

Bulgular ve Sonuç: Bu çalışmada subklinik mastitisli Saanen keçilerinden alınan 280 adet süt numunesinden 138 (% 49.3) adet bakteriyel izolasyon yapılmıştır. Bakteriyel izolatlardan 90 (% 32.1) adedi *S .aureus* olarak identifiye edilmiştir. Bütün *S. aureus* (n=90) suşları antimikrobiyel duyarlılık testine tabi tutulmuştur. *S. aureus* izolatları, oksitetrasikline karşı % 100, ampisiline karşı % 91 ve vankomisine karşı % 60 oranında dirençlilik göstermiştir. *S. aureus* etkeni, subklinik mastitiste etiyolojik ajan olarak sayıca daha fazla bulunmuştur ve novobiosin, Saanen keçilerinde görülen subklinik mastitis hastalığında söz konusu etkene karşı en etkili antibiyotik olarak tespit edilmiştir.

Anahtar kelimeler: Saanen Keçisi, Subklinik Mastitis, S. aureus, İdentifikasyon, Antimikrobiyel dirençlilik

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Introduction

Mastitis is generally described as the inflammation of mammary gland. The traumatical, pathological and bacteriological changes in goat mammary glands causes the physical and chemical changings in milk and causes mastitis (Shearer and Harris, 2003).

Mastitis is the total or partial inflammation of the mammary gland provoked by one or more pathogenic microorganisms, which can appear either in clinical or subclinical forms (Schalm et al., 1971). The subclinical one is a form of the disease in which there is neither detectable change in the udder nor observable abnormalities in milk. However, the presence of microorganisms in milk usually can be demonstrated by microbial culture, and inflammatory changes in the milk can be detected by special methodologies such as somatic cell count. *Staphylococcus* spp. are the most frequently diagnosed microorganisms responsible for intramammary infection in goats and sheeps (Contreras et al., 2007).

The most important mastitis pathogen is *Staphylococcus aureus*. Gangrene should be seen in severe cases. Bubbles in milk secretion should be seen with the affiliation of gas producing bacteria. Death should be formed suddenly or in several days according to severity of infection. Some animals should be treated by suspension of necrotic tissue (Shearer and Harris, 2003).

In dairy goats with subclinical mastitis, *S. aureus* make up 4.1% to 18.0% of the isolated pathogens from milk samples. The average prevalence of subclinical mastitis in dairy goat's farms is between 20.0%–35.0%, and results in significant economic losses due to reduction in milk production and poor milk quality (Contreras et al., 2003).

The intramammary administration of antibiotics used on farms has increased, as it was proved to be effective for treating subclinical mastitis in dry small ruminants (Poutrel et al., 1997; Santis et al., 2001). The efficacy of intramammary antibiotic treatment could be compromised by staphylococci that produce biofilms in the udder. The widespread use of antibiotics on dairy farms, could lead to the selection and to the emergence of antibiotic resistant bacterial strains (Walther et al., 2006).

The aim of this study was to establish the resistance of antimicrobial agents to *S. aureus* isolated from Saanen goats with subclinical mastitis.

Material and Method

The milk samples were collected from one Saanen goats flock in the Western Turkey. A clinical examination of half-udders was conducted in order to exclude animals with signs of clinical mastitis (Donovan et al., 1992). A total of 280 half-udder milk samples (140 Saanen goats) were collected in a single sampling time in a flock during the early lactation period. The first few streams of fore milk were discarded, and duplicate half-udder milk samples were aseptically collected into sterile tubes after cleaning and disinfection of each teat end. One sample (10 mL) was used for bacteriological analysis. The milk samples were stored at +4 ℃, and bacteriological examinations were carried out within 6 and 24 hours after sampling.

S. aureus Identification from Saanen Milk Samples

The milk samples were incolated to 5 % sheep blood agar (Difco) incubated at 37°C for 24-48 hours. After that, the morphology, pigmentation and haemolysis of grown colonies were investigated with Gram's staining method. The identification was made to susceptible colonies according to criteria below. Gram positive strains were performed with catalase reaction by 3% H_2O_2 and positive microorganisms were evaluated as Micrococcaceae family (Koneman et al., 1997). Coagulase test with 1/5 diluated citrated rabbit plasm were performed to the strains separated as *Staphylococci* and the strains were separated as coagulase positive and negative. The microorganism examined were grown in 1 ml tryptic soy Broth (Oxoid) and inoculated to Mueller-Hinton agar. Bacitracin discs (Oxoid) (0.04 U/ml) were placed onto inoculation zone. After incubation at 37°C for 18 hours, the resistant strains to discs were evaluated as *Staphylococci* (Koneman et al., 1997). Coagulase positive *Staphylococci*, were identified as *S. aureus* according to urease activity, hemolysin, mannitol, oxidase and DNAse activity (Koneman et al., 1997; Holt et al, 1994). All isolates were identified using API ID32 STAPH system (bioM´erieux).

Antimicrobial Agents and Minimal Inhibitory Concentration (MIC).

On each strain the MICs of ten antibiotics used in human and veterinary medicines were determined. The antibiotics tested were ampicillin (AMP), cefoperazone (CFP), ceftriaxone (CRO), kanamycin (K), novobiocin (NV), oxytetracycline (OT), and vancomycin (VA). The MICs were determined by the broth microdilution method (CLSI, 2006a; NCCLS, 2002a) using cation-adjusted Mueller-Hinton broth (CAMHB, Oxoid, Basingstoke, UK). Each antimicrobial agent, in powder form (Sigma-Aldrich-Fluka), was weighed and dissolved in an appropriate solvent (CLSI, 2006a; NCCLS, 2002a), thus obtaining a stock solution (2,560 μg/mL). Stock solutions were stored at −80 ℃ until used. From each stock solution, 12 serial twofold working dilutions in deionized water (only for AMP, the diluents were phosphate buffer, pH 6.0, 0.1 mol/L) was prepared according to CLSI standard protocols, and the antimicrobial agent final concentrations in each microplate ranged between 0.06 and 256 μg/mL. Each strain stored at −80 ℃ until testing were subcultured twice on BHI agar (Oxoid) before inoculum preparation. Two or more identical colonies were picked from BHI plates after overnight incubation and suspended in saline solution (0.85% w/v) to match a McFarland 0.5 turbidity standard. Each suspension was further diluted 1:100 in CAMHB in order to achieve the adequate inoculum concentration (10^6 cfu/mL). Fifty microliters of the final suspension were inoculated into the wells of microtiter plates, which also contained 50 μL of the antimicrobial agent, so that the final inoculum density on test plates contained 5 x 10^5 cfu/mL in each well. Reference strains, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212, were used for quality control. Each microplate was then incubated at 35±2℃ under aerobic environment for 20 hours. The susceptibility of each strain to the antimicrobial agents was then defined by comparing the results to those of the breakpoint values (CLSI, 2006b; NCCLS, 2002b; Thornsberry et al., 1997). The MICs range and mode, MIC_{50} and MIC_{90} of each antimicrobial agent were also determined.

Results

In this study, bacterial isolation was made from 138 (49.3%) out of 280 milk samples taken from subclinical mastitic milk of Saanen goat. No isolation was detected from remaining 142 (50.7%) samples. Bacterial strains were identified as *S. aureus* 90 (32.1%), coagulase negative *Staphylococcus* 40 (14.2%), *Klebsiella pneumonia* 4 (1.4%) and *E. coli* 4 (1.4%). All isolates

Table 1. MICs values of *S. aureus* isolates and the percentage of antimicrobial resistance.
Tablo 1. *S. aureus* izolatlarının MIC değerleri ve antimikrobiyal dirençlilik yüzdeleri.

	S. aureus				
Antimicrobial Agent	**MIC (µg/mL) breakpoints**	**MIC Range (µg/ml)**	**MIC_{50} (µg/ml)**	**MIC_{90} (µg/ml)**	**Antimicrobial Resistance (%)**
Ampicillin	≤0.25–≥0.5	≤0.06 – 64	4.0	8.0	91
Cefoperazone	≤16.0–≥64.0	0.25 - ≥256	8.0	32.0	10
Ceftriaxone	≤8.0–≥64.0	0.25 - ≥256	2.0	16.0	11
Kanamycin	≤16.0–≥64.0	2.0 - ≥256	8.0	64.0	30
Novobiocin	≤4.0	0.125 – 4.0	1.0	4.0	0
Oxytetracycline	≤4.0–≥16.0	16.0 - ≥256	32.0	128.0	100
Vancomycin	≤4.0–≥32.0	0.125 - ≥256	16.0	64.0	60

were confirmed by API Staph and API 20 E kits.

All *S. aureus* isolates (n=90) were subjected to antimicrobial susceptibility testing. The MICs values of *S. aureus* strains isolated from subclinical mastitic milk of goat and antimicrobial resistance to different antibiotics are shown in Table 1.

All *S. aureus* strains were resistant to oxytetracycline. Eighty-two (91%) of 90 *S. aureus* strains were resistant to ampicillin and fifty-four (60%) of 90 *S. aureus* strains were resistant to vancomycin.

All *S. aureus* strains were susceptible to novobiocin (100%). *S. aureus* strains were susceptible to ceftriaxone (91%), cefoperazone (90%) and kanamycin (70%).

In this study, ampicillin and oxytetrecycline MIC_{90} values were higher than the breakpoint values.

Discussion

Subclinical mastitis in goats is mainly of bacterial origin (Bergonier et al. 2003). In this study, *S. aureus* was identified higher than the other bacteria. *S. aureus* was identified 90 (32.1%) out of 280 Saanen mastitic milk samples.

The prevalence of goat mastitis was found as 38.2% in New York (Smith and Roguinsky, 1977) and 8.2% to 54.4% of which were in a group of California goat dairies (East et al., 1987). Studies of goat mastitis conducted in Spain, England, and France have reported prevalences of 18%, 36%, and 32.6%, respectively (Poutrel and Lerondelle, 1983; Manser, 1986; Contreras et al., 1995). Our results are similar to the other research (Smith and Roguinsky, 1977; Manser, 1986; Contreras et al., 1995).

S. aureus is the most significant pathogen of the caprine mammary gland. *S. aureus* infections may be subclinical, chronic, acute, or gangrenous in their most severe form. The organisms are often resistant to antibiotic treatment due to their ability to exist both intracellularly and in L-forms, and due to their location within microabscesses in the udder (Sears et al., 1987; Smith and Sherman, 1994).

In this study, all *S. aureus* strains were resistant to oxytetracycline. Eighty-two (91%) of 90 *S. aureus* strains were resistant to ampicillin and fifty-four (60%) out of 90 *S. aureus* strains were resistant to vancomycin. The MIC range of vancomycin is between 0.125 - ≥256$\mu g/ml$ and MIC_{90} value of vancomycin ranged 64.0 $\mu g/ml$.

When these strains are transferred from animals to humans, they could increase the spreading of vancomycin-resistant *S. aureus* (VRSA) strains.

Oxytetracycline and ampicillin were less effective than the other antimicrobial agents. In previous studies, a number of authors have observed a marked variability in the susceptibility of both this microorganism to tetracycline, as it ranged between 10.0% and 100.0% (Bochev and Russenova, 2005; da Silva, 2004).

Moroni et al. (2005), have found a markedly greater prevalence of ampicillin resistant *S. aureus* (67.9%). These findings are consistent with ampicillin sensitivity to the penicillinases, frequently produced by *Staphyloccus* spp. In this study, *S. aureus* isolates were 91% resistant to ampicillin. The MIC values of ampicillin ranged between ≤0.06 and 64 $\mu g/ml$.

The cephalosporins showed a strong activity with regard to *S. aureus* isolates. *S. aureus* strains were susceptible ceftriaxone (91%), cefoperazone (90%). The MIC range of cefoperazone is between 0.25 - ≥256 $\mu g/ml$ and MIC_{90} value of cefoperazone ranged 32.0 $\mu g/ml$.

This study, *S. aureus* isolates were susceptible to novobiocin (100%). This specialty is of interest in the taxonomy for bacterial typing since it is also well related with pathogenic activity (Deinhofer and Pernthaner, 1995).

In conclusion, we can conclude that the subclinical mastitis in Saanen goats has a prevalence of 32.1%, and this study confirms that *S. aureus* is the most common pathogens associated with subclinical mastitis in Saanen goats. The *S. aureus* isolates were resistant to ampicillin and oxytetracycline. This situation may be due to misuse of antibiotics, because frequently use of the same antibiotics may lead to antibiotic resistance. Moreover, prevalence was high in those goats where sanitation conditions were poor and teat injury was present in Saanen goats. It was also revealed that *S. aureus* are more in number as causative agents in subclinical mastitis and novobiocin is the most effective antibiotic in Saanen subclinical mastitis.

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