

Identification and Antimicrobial Susceptibility of Subclinical Mastitis Pathogens Isolated from Hair Goats' Milk

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Abstract: Aim of this study, was to identify the pathogens responsible for subclinical mastitis in hair goats and to determine their susceptibility to antimicrobial drugs. Total 700 milk samples from clinically healthy half udders of 350 lactating hair goats were collected and examined. The isolates were identified by conventional methods. Antibiotic susceptibility test was performed using disk diffusion method. Of the 700 milk samples examined, 60 (8.6%) were subclinically infected. Coagulase Negative Staphylococci (CNS), *Streptococcus* sp. and *Staphylococcus aureus* were the main species of microorganisms isolated. The CNS were the most common pathogen in this study with an prevalence of 50%. CNS were generally resistant to lactam antibiotics, while *S. aureus* and *Streptococcus* sp. were susceptible to lactams. Although, CNS and *S. aureus* were susceptible to aminoglycosides, fluoroquinolones, erythromycin, sulphamethoxazole/trimethoprim, lincomycin, oxytetracycline and florfenicol, *Streptococcus* sp. were susceptible to lactams, aminoglycosides, sulphamethoxazole/trimethoprim, erythromycin, oxytetracycline and florfenicol. As results, it may be stated that antimicrobial drug susceptibility tests in subclinical mastitis of the hair goats should be done before the treatments.

Key words: Goat, subclinical mastitis, pathogens, antibiotics, susceptibility, milk

INTRODUCTION

Turkish hair goat are raised in all parts of Turkey, particularly in the mountainous and brushy areas of Mediterranean, Aegean and southeastern Anatolian Regions. Hair goats raised in hilly and mountainous regions depend for their nutrition. The body size is considered large. Body weight, lactation milk yield, lactation length, milk fat, hair production, birth rate and twinning rate of does 40-45 kg, 60-70 kg, 150-160 day, 4-5%, 0.5-0.6 kg, 80-85 and 5-15%, respectively. It is ability to make use of natural resources (pastures). Although milk yield of hair goats is low, their milk and milk products are important source of income for region public (Yalcin, 1986).

The dairy goat industry is rapidly gaining in importance throughout the world in recent years. Therefore, any factor that adversely affects the quantity and quality of goat milk is of great financial interest. Milk quality is mainly affected by bacterial contamination of

the mammary gland, which causes clinical or subclinical mastitis (Boscos *et al.*, 1996). Mastitis in the goat is mainly subclinical (Contreras *et al.*, 1995, 1999; McDougall *et al.*, 2001). Subclinical mastitis reduces milk production and is one of the factors for losing of kids. Subclinical mastitis also, has a negative impact on hygienic milk quality and it is responsible for major economic losses (Maisi, 1990a; Contreras *et al.*, 1996, 2003).

Diagnosis of subclinical mastitis in goats is not easy and direct bacteriological assay is the recommended method (Maisi and Riipinen, 1988; Maisi, 1990a; Fthenakis, 1995; Gonzalez-Rodriguez and Carmenes, 1996). Although some diagnostic tests (CMT, NAGase, SCC, etc.) (Poutrel and Lerondelle, 1983; Maisi and Riipinen, 1988; Maisi, 1990b) are used the determination of subclinical mastitis, bacteriological culture is the gold standard in the diagnosis of subclinical mastitis (Poutrel and Lerondelle, 1983; Sanchez *et al.*, 2004). Definitive detection of infected goats relies on positive

culture of pathogens from aseptically collected milk samples (McDougall *et al.*, 2001). Subclinical mastitis in goats are mainly of bacterial origin (Bergonier *et al.*, 2003). *Staphylococcus* sp. are the main aetiological agents of clinical and subclinical mastitis in goats (Hunter, 1984; Poutrel, 1984; Contreras *et al.*, 1995; Ajuwape *et al.*, 2005). While, *S. aureus* and *Escherichia coli* are most commonly isolated pathogen from the clinical mastitis (Ameh *et al.*, 1993; Ameh and Tari, 2000; Contreras *et al.*, 2003), Coagulase Negative Staphylococci (CNS) are the most frequently isolated pathogens from the subclinical goat mastitis (Ndegwa *et al.*, 2001; McDugall *et al.*, 2002; Bergonier *et al.*, 2003; Contreras *et al.*, 2003). In the treatment of mastitis, intramammary and/or parenteral antibiotics (lactams, macrolides, fluoroquinolones) are used. However, no controlled studies are available on the efficacy of parenteral or intramammary antibiotherapy (Bergonier *et al.*, 2003). Wrong or incomplete treatments of infections may cause an antibiotic resistance. In addition this, there is a few data available in the literature on the susceptibility of goat mastitis pathogens (Da Silva *et al.*, 2004; Moroni *et al.*, 2004).

Aim of this study, was to identify the pathogens responsible for subclinical mastitis in hair goats and to determine their susceptibility to antimicrobial drugs.

MATERIALS AND METHODS

Sample collection and microbiological analysis: Total 700 milk samples from clinically healthy half udders of 350 lactating hair goats were collected and examined. Before sampling the teat ends were cleaned with alcohol swabs and allowed to dry. The 1st few streams were discarded and then 5 mL of secretion was collected in sterile tubes. Samples were cooled and immediately transported to the laboratory. From each milk sample, 0.1 mL was plated on Columbia blood agar medium (Oxoid Ltd, Hampshire, UK), containing 5% of sheep blood and 0.1% of esculin and incubated at 37°C for 48 h. The isolates were identified by conventional methods, including Gram staining, colony morphology, haemolysis, tests for catalase, clumping factor, coagulase, DNase, acetoin and anaerobic fermentation of mannitol. All the tests were performed as described by Koneman *et al.* (1992). All the isolates were stored at -20°C in trypticase soy broth containing 10% of glycerol. Prior to the testing, the isolates were twice serially cultured on Columbia blood agar medium, containing 5% of sheep blood, for 24 h at 37°C under aerobic conditions. In this study, the term subclinical mastitis is used for cases, in which milk samples were microbiologically positive 5 or more colonies of a single organism (Fthenakis, 1994; Contreras *et al.*, 1996; Sanchez *et al.*, 2004).

Antibiotics susceptibility test: In the study, antibiotic susceptibility test was performed on the mostly isolated pathogens (CNS, *S. aureus*, *Streptococcus* sp). Antibiotic susceptibility test was performed using disk diffusion method on Mueller-Hinton agar (Oxoid) according to the National Committee of Clinical Laboratory Standards. Ten colonies from the Columbia blood agar medium, incubated at 37°C for 24 h, were suspended in 2 mL of sterile saline to a density approximately equal to McFarland Opacity Standard No. 0.5. A dry sterile cotton wool swab was placed in the suspension and excess liquid was expressed against the inside of the tube. The bacterial suspension was inoculated onto Mueller-Hinton agar with the swap in such a way that the whole surface of the agar was covered.

The antibiotic disks, containing the following antibiotics: penicillin G (10 U, Oxoid), ampicillin (10 µg, Oxoid), amoxycillin (25 µg, Oxoid), ampicillin/sulbactam (20 µg, Oxoid), amoxicillin/clavulanic acid (30 µg, Oxoid), cloxacillin (25 µg, Oxoid), cefuroxime (30 µg, Oxoid), cefoperazone (30 µg, Oxoid), cefoperazone/sulbactam (105 µg, Oxoid), neomycin (30 µg, Bioanalyse), gentamycin (10 µg, Oxoid), enrofloxacin (5 µg, Oxoid), danofloxacin (5 µg, Pfizer), sulphamethoxazole /trimethoprim (25 µg, Oxoid), erythromycin (15 µg, Oxoid), lincomycin (10 µg, Oxoid), oxytetracycline (30 µg, Oxoid) and florfenicol (30 µg, Oxoid). These disks were dispensed on the surface of the medium and incubated aerobically at 37°C for 24 h. The results were recorded as resistant or susceptible by measurement of inhibition zone diameter according to the interpretive standards of National Committee for Clinical Laboratory Standards.

RESULTS

On cultural examination of the 700 milk samples obtained from clinically healthy half udders, pathogens were detected in 60 samples. Prevalence of subclinical mastitis was 8.6% of all udder half samples examined. CNS (n: 30), *S. aureus* (n: 7), *Streptococcus* sp. (n: 9), other bacteria (n: 3, *Corynebacterium* sp., *E. coli*, *Klebsiella* sp.) and *Candida* sp. plus yeast (n: 11) are isolated as pathogens. CNS were the predominant organisms isolated (50%), followed by *Streptococcus* sp. (15%), *S. aureus* (11.7%) and other pathogens (23.3%).

The antibiotic susceptibility rate of bacteria isolated from clinically normal hair goats milks are detailed in Table 1. CNS showed the highest resistance to lactam antibiotics except for cloxacillin and cefoperazone/sulbactam, while *S. aureus* and *Streptococcus* sp. showed the highest susceptibility to lactams. CNS and *S. aureus* were susceptible to aminoglycosides, fluoroquinolones, sulphamethoxazole/trimethoprim, erythromycin,

Table 1: Antibiotic susceptibility of CNS, *S. aureus* and *Streptococcus* sp. isolated from clinically healthy half udders of hair goats

Antibiotics	CNS (n = 30)				<i>S. aureus</i> (n = 7)				<i>Streptococcus</i> sp. (n = 9)			
	Susceptible		Resistant		Susceptible		Resistant		Susceptible		Resistant	
	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)	n	(%)
Penicillin G	10	33.3	20	66.7	6	85.7	1	14.3	9	100.0	0	0.0
Ampicillin	10	33.3	20	66.7	6	85.7	1	14.3	9	100.0	0	0.0
Amoxicillin	10	33.3	20	66.7	6	85.7	1	14.3	9	100.0	0	0.0
Ampicillin/sulbactam	10	33.3	20	66.7	7	100.0	0	0.0	9	100.0	0	0.0
Amoxycillin/clavulanic acid	12	40.0	18	60.0	7	100.0	0	0.0	9	100.0	0	0.0
Cloxacillin	27	90.0	3	10.0	7	100.0	0	0.0	6	66.7	3	33.3
Cefuroxime	7	23.3	23	76.7	4	57.1	3	42.9	6	66.7	3	33.3
Cefoperazone	10	33.3	20	66.7	6	85.7	1	14.3	5	55.6	4	44.4
Cefoperazone/sulbactam	19	63.3	11	36.7	7	100.0	0	0.0	8	88.9	1	11.1
Neomycin	28	93.3	2	6.7	6	85.7	1	14.3	6	66.7	3	33.3
Gentamycin	30	100.0	0	0.0	7	100.0	0	0.0	6	66.7	3	33.3
Enrofloxacin	30	100.0	0	0.0	7	100.0	0	0.0	3	33.3	6	66.7
Danofloxacin	29	96.7	1	3.3	7	100.0	0	0.0	1	11.1	8	88.9
Sulphamethoxazole/trimethoprim	30	100.0	0	0.0	7	100.0	0	0.0	7	77.8	2	22.2
Erythromycin	24	80.0	6	20.0	6	85.7	1	14.3	6	66.7	3	33.3
Lincomycin	20	66.7	10	33.3	6	85.7	1	14.3	4	44.4	5	55.6
Oxytetracycline	21	70.0	9	30.0	7	100.0	0	0.0	6	66.7	3	33.3
Florfenicol	30	100.0	0	0.0	7	100.0	0	0.0	9	100.0	0	0.0

lincomycin, oxytetracycline and florfenicol. Although, *Streptococcus* sp. were susceptible to lactams, aminoglycosides, sulphamethoxazole/trimethoprim, erythromycin, oxytetracycline and florfenicol, they were resistance to fluoroquinolones.

DISCUSSION

A large percentage of non-clinical caprine milk samples was found to be infected with mastitis pathogens (Kalogridou-Vassiliadou, 1991). Mastitis in the goat is mainly subclinical (Contreras *et al.*, 1995, 1999; McDougall *et al.*, 2001).

Subclinical mastitis in dairy small ruminants are mainly of bacterial origin (Bergonier *et al.*, 2003). Information concerning antimicrobial susceptibility of subclinical mastitis pathogens isolated from hair goats is limited.

In the present investigation, prevalence of subclinical mastitis was 8.6% in the hair goats. Sanchez *et al.* (2004) reported similar prevalence, while, Contreras *et al.* (1995, 1996), Boscós *et al.* (1996) and McDougall *et al.* (2001) reported higher prevalence than present investigation. It was stated that prevalence of subclinical mastitis in the different goat herds have been ranged from 7-34% the glands (Contreras *et al.*, 1995). There is large between herd variations. This has been attributed to the influence of factors such as breed differences, age and parity of the animals, stage of lactation, different hygiene and management practices followed on each farm and the milking method (East *et al.*, 1987; Contreras *et al.*, 1995; Boscós *et al.*, 1996; McDougall *et al.*, 2002; Moroni *et al.*, 2005c).

Staphylococcus sp. are the main aetiological agents of clinical and subclinical mastitis in goats (Hunter, 1984; Poutrel, 1984; Contreras *et al.*, 1995; Ajuwape *et al.*, 2005). In this study, CNS, *Streptococcus* sp. and *S. aureus* were the main species of bacteria isolated (Table 1). CNS were the most common pathogen in this study with an prevalence of 50%. This finding is in agreement with that reported by Hunter (1984), East *et al.* (1987), Boscós *et al.* (1996), Ndegwa *et al.* (2001), McDougall *et al.* (2002), Contreras *et al.* (2003), Sanchez *et al.* (2004) and Moroni *et al.* (2005c).

The antibiotic susceptibility rate of bacteria isolated from clinically normal hair goats milks are detailed in Table 1. lactam antibiotics are used in the treatment of mastitis in the goats (Moroni *et al.*, 2004). In the current study, CNS showed the resistance to lactam antibiotics involved lactamase inhibitors plus penicillins, but CNS were susceptible to cloxacillin and cefoperazone/sulbactam, a 3rd generation cephalosporin antibiotics. It was reported that some CNS species were resistance to penicillin G in the caprine (Da Silva *et al.*, 2004) and bovine mastitis (Turutoglu *et al.*, 2006), while CNS were susceptible to beta-lactam antibiotics except for cefoperazone in the subclinical mastitis of goats (Moroni *et al.*, 2004, 2005a). This study result may indicate that CNS frequently possess resistance to penicillin and cephalosporin antibiotics. This may be due to misuse of antibiotics, because frequently and incomplete antibiotics treatments may cause resistance in the bacteria (Tras *et al.*, 2007). In the current study, CNS were susceptible to aminoglycosides, fluoroquinolones, sulphamethoxazole/trimethoprim, erythromycin, lincomycin, oxytetracycline and florfenicol. It is well

known that aminoglycosides and fluoroquinolones highly effective to *Staphylococcus* sp. (Tras *et al.*, 2007) and erythromycin, tetracycline and sulphamethoxazole/trimethoprim susceptibility were also reported in the goats (Da Silva *et al.*, 2004). On the contrary to this, erythromycin and tetracycline resistance were reported in the CNS (Moroni *et al.*, 2004; Moroni *et al.*, 2005a). It is expected that CNS show very variable sensitivity to the antibiotics because CNS possess very different species.

In the present study, *S. aureus* was susceptible to all tested antibiotics. Aminoglycoside and fluoroquinolone antibiotics are very effective to *Staphylococcus* sp. (Tras *et al.*, 2007). Lactams (Da Silva *et al.*, 2004; Moroni *et al.*, 2005b), erythromycin, tetracycline and sulphamethoxazole/trimethoprim (Da Silva *et al.*, 2004) susceptibilities were reported in the goat mastitis. In addition, lactams, aminoglycosides and fluoroquinolones susceptibilities were reported in the ewes (Fthenakis, 1998; Pengov and Ceru, 2003) and bovine mastitis (Fthenakis, 1998; Turutoglu *et al.*, 2006).

In the current study, *Streptococcus* sp. were susceptible to lactams, aminoglycosides, sulphamethoxazole/trimethoprim, erythromycin, oxytetracycline and florfenicol, while they were resistance to fluoroquinolones. Environmental streptococci are frequently caused to mastitis and lactam antibiotics are highly effective to *Streptococcus* sp., while, fluoroquinolones are less effective (Tras *et al.*, 2007). Some *Streptococcus* strains were susceptible to lactams and chloramphenicol in the cow mastitis (Guerin-Fauble *et al.*, 2002). Different results obtained from the antibiotic susceptibility studies may be mainly due to misuse of antibiotics and differences of bacterial strains.

CONCLUSION

Mastitis pathogens might be isolated from clinically healthy half udders of hair goats and antimicrobial drug susceptibility tests may be beneficial in the treatment of subclinical mastitis in the hair goats before the treatments because of observation the regional susceptibility to antimicrobial drugs.

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