

Drug resistance in isolated bacteria from milk of sheep and goats with subclinical mastitis in Shahrekord district

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Summary

Four-hundred milk samples from half udders of 20 flocks of native breed goats were aseptically collected. The samples were examined by California mastitis test (CMT). Twenty-one (5.25%) of 400 were CMT-positive. The CMT-positive samples were then cultured and the following bacteria were isolated: *Staphylococcus aureus* in 3 (14.28%), CNS (coagulase-negative staphylococci) in 14 (66.66%), streptococci in 4 (19.04%) and *Mycoplasma* spp. in 11 (52.38%) samples. The isolated staphylococci were examined to evaluate their resistance to different antibiotics by disc diffusion method. CNS resistance to amikacin was 78.5%, to penicillin was 50%, to tetracycline 50%, to ampicillin 42.8%, and to doxycycline was 28.5%. Four-hundred native breed sheep milk samples were taken from 19 flocks in the same conditions. The samples were examined by CMT test. 19 (4.75%) of 400 were CMT-positive. The CMT-positive samples were cultured and the following bacteria were isolated: *S. aureus* in 2 (10.5%), CNS in 7 (36.8%), streptococci in 2 (10.5%), *Pasteurella* in 1 (5.26%) and *Mycoplasma* in 9 (47.37%) samples. The isolated staphylococci were examined for antibiotic susceptibility. CNS resistance to amikacin was 42.8%, and to tetracycline, ampicillin, oxytetracycline and penicillin was 14.3%. Both isolates of *S. aureus* were resistance to penicillin.

Key words: Subclinical mastitis, Sheep, Goat, Antibigram

Introduction

Dairy sheep and goats farming is an important source of animal protein in the central Iran where land can not support dairy cattle farming. In these areas, the available animal food is poor and mainly comprises of crop residues, weeds and shrubs. Dairy sheep/goats, with their efficient digestive system, small body size and low food intake are thus cheaper and more economical to keep in these areas. Clinical mastitis in sheep has generally been considered as a main source of finance loss but the economic loss caused by subclinical mastitis is also important. Clinical ovine mastitis is typically gangrenous and causes death. Subclinical mastitis results in decreased milk yield in sheep in consequence growth

retardation and higher mortality rate among suckling lambs.

Subclinical mastitis is characterized by no visible sign of disease by apparently normal milk, with an increase in somatic cell count (SCC) that is bacteriologically positive. Diagnosis of mastitis needs special screening tests, such as the California mastitis test (CMT). The primary cause of subclinical mastitis in sheep/goats is *Staphylococcus* spp., *Streptococcus* spp. and *Micrococcus* spp. (Waston and Buswell, 1984; Egwu *et al.*, 1994; Contreras *et al.*, 1995; Boscov *et al.*, 1996; Bahout and Balata, 1998; Ndegwa *et al.*, 2000).

The objective of this survey was to isolate the udder pathogenic bacteria and determination of their antibiotic resistance profile in milk samples of sheep/goats with

subclinical mastitis in Shahrekord region, central Iran.

Materials and Methods

A total of 800 milk samples—400 from half udders of native breeds lactating sheep and 400 samples from goats—were collected aseptically at mid-lactation from 19 sheep and 20 goat flocks, through a cluster sampling method. The study was carried out over a six-month period starting from April 2003. Collected milk samples were followed by CMT test. Milk from the affected mammary glands (CMT ≥ 2) were collected in sterile vials and kept under refrigeration during transport to the laboratory for microbiological analysis. A half udder was considered infected when a single known pathogenic bacterium was isolated (Casadevall and Pirofski, 2000). Isolation and identification of bacteria from milk samples were performed according to the generally-accepted rules (Quinn *et al.*, 2002).

Briefly, samples were surface plated and incubated on sheep blood agar, MacConkey agar for 48 hrs at 37°C, PPLO agar containing penicillin and thallium acetate for five days in 5-7% CO₂ and 37°C. The Christie, Atkins, Munch-Petersen (CAMP) test with *S. aureus* and coagulase test were also performed (Quinn *et al.*, 1999).

Biochemical identification of the isolated bacteria was achieved using the accepted rules (Quinn *et al.*, 2002). Susceptibility to antimicrobial agents was determined for isolated bacteria other than *Mycoplasma* and streptococci by the disk diffusion method on Mueller-Hinton agar following the National Committee for Clinical Laboratory Standards guidelines (NCCLS 2000).

Results

Visual observation and palpation of the mammary gland halves and macroscopic examination of the milk revealed no abnormalities. Tables 1 to 4 show the isolated bacteria and their antibiotic resistance patterns from CMT-positive sheep and goats. Twenty-one (5.25%) goats and 19

(4.75%) sheep were CMT-positive.

From the bacteria isolated from goat milk samples, 14 (66.66%) were coagulase-negative *Staphylococcus* (CNS), three (14.28%) were *S. aureus*, four (19.04%) non-hemolytic CAMP-negative streptococci, and 11 (52.38%) *Mycoplasma* spp. Six isolates of *Mycoplasma* were accompanied with CNS isolations.

Table 1: Bacterial isolations from milk of 19 CMT-positive sheep

Isolated bacteria	Positive samples n (%)	Negative samples n (%)
<i>Staphylococcus aureus</i>	2 (10.5%)	17 (89.5%)
Coagulase-negative staphylococci	7 (36.8%)	12 (63.2%)
<i>Pasteurella</i> spp.	1 (5.26%)	18 (94.74%)
<i>Mycoplasma</i> spp.	9 (47.37%)	10 (52.63%)
<i>Streptococcus</i> spp.	2 (10.5%)	17 (89.5%)

Table 2: Antibiotic resistance of 7 isolates of CNS from milk of 19 CMT-positive sheep

Antibiotics	Number of susceptibles	Number of resistants
Tetracycline	3	1
Streptomycin	5	0
Ciprofloxacin	4	0
Carbenicillin	-	-
Amikacin	2	3
Ampicillin	3	1
Gentamicin	4	0
Doxycycline	4	1
Chloramphenicol	2	0
Penicillin	0	1

Table 3: Bacterial isolates from milk of 21 CMT-positive goat

Isolated bacteria	Positive samples n (%)	Negative samples n (%)
<i>Staphylococcus aureus</i>	3 (14.28%)	18 (85.71%)
Coagulase-negative staphylococci	14 (66.66%)	7 (33.33%)
<i>Mycoplasma</i> spp.	11 (52.38%)	10 (47.61%)
<i>Streptococcus</i> spp.	4 (19.04%)	17 (80.95%)

Table 4: Antibiotic resistance of 14 isolates of CNS from milk of 21 CMT-positive goat

Antibiotics	Number of susceptibles	Number of resistants
Streptomycin	13	0
Ciprofloxacin	13	0
Amikacin	1	11
Ampicillin	5	6
Gentamicin	13	0
Doxycycline	7	4
Chloramphenicol	10	0
Penicillin	2	7
Tetracycline	3	7

From the bacteria isolated from sheep milk samples, seven (36.8%) were CNS, two (10.5%) were *S. aureus*, two (10.5%) non-hemolytic CAMP-negative streptococci, one (5.26%) *Pasteurella* and nine (47.37%) were *Mycoplasma* spp. Three isolates of *Mycoplasma* were accompanied with CNS isolations.

In goat isolates, CNS resistance to amikacin was 78.5%, to penicillin was 50%, to tetracycline 50%, to ampicillin 42.8%, and to doxycycline was 28.5%. All isolates of *S. aureus* were susceptible to ciprofloxacin, carbenicillin and gentamicin; two isolates were resistant to tetracycline, ampicillin and amikacin.

In sheep isolates, CNS resistance to amikacin was 42.8%. The resistance to tetracycline, ampicillin, oxytetracycline and penicillin was 14.3%. Both isolates of *S. aureus* were resistance to penicillin.

Discussion

Intra-mammary bacterial infection (of CMT-positive samples) was 5.25% in lactating goats and 4.75% in ewes. Although this was similar to some previous reports (Kalogridou *et al.*, 1992) it differed from some others (Boscós *et al.*, 1996; Nazer and Jafari, 2000; Ndegwa *et al.*, 2000). The frequency of bacterial species isolated from clinically-normal sheep/goats milk is affected by factors such as breed differences, different hygiene and management practices followed in each farm, age and parity of the animal and type of milking (East *et al.*, 1987; Boscós *et al.*, 1996).

CNSs are probably the first group of important microorganisms, responsible for inducing subclinical mastitis in sheep and goats (Boscós *et al.*, 1996; Ndegwa *et al.*, 2000). Although streptococci are the species more frequently identified, infections by these microorganisms in animal species have been associated with hand milking (Gonzalez-Rodriguez *et al.*, 1995).

In our survey, the most frequent bacterial group in both sheep and goats was CNS. *Mycoplasma* is most often a usual finding in normal milk. Only large concentration of this microorganism in mastitic milk is evidence that it possess a

pathogenic potential (Da Massa, 1983).

Antimicrobial susceptibility patterns should be identified for CNS, as it varies in different geographical regions (Gentilini *et al.*, 2002).

Currently, streptomycin, ciprofloxacin and gentamicin are very active against isolated CNSs. Similar results were found in Argentina (Frigerio *et al.*, 1995).

Our results are in agreement with other reports that confirm the role of CNS in subclinical mastitis of sheep and goats (Boscós *et al.*, 1996; Shawkat and Lafinabil, 1998; Ndegwa *et al.*, 2000).

The high resistance of CNSs isolated from sheep and goats to amikacin could predict low success of treatment of subclinical mastitis with this antibiotic. Antimicrobial susceptibility patterns should be identified for CNS, as current susceptibility data are necessary to select appropriate antibiotics for a successful treatment. Based on the present results, chloramphenicol, ciprofloxacin, streptomycin or gentamicin are currently good for treating future clinical cases.

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