

## Characteristics of Staphylococci isolated from mastitic goat milk in Iranian dairy herds

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### Abstract

One hundred and fifteen cases of sub clinical mastitis were detected in a study on 510 goats from 5 herds in west central, Iran. From positive milk samples, 23 *Staphylococcus* spp. strains were isolated. Fourteen isolates were determined as *Staphylococcus aureus* (12.17%), and the other 9 (7.82%) as *Staphylococcus epidermidis*. Eleven and six isolates of *S. aureus* and *S. epidermidis* produced combined form of hemolysins  $\alpha/\beta/d$ , while  $\beta/d$  hemolysins produced by 2 and 3 isolates respectively. Only one isolate of *S. aureus* produced single type d hemolysin. The sensitivity of all strains to 10 chemotherapeutics was tested through the disk diffusion method, 6 strains (26.08%) were determined as methicillin-resistant: out of them 4 isolates were *S. aureus* and 2 *S. epidermidis*. *S. aureus* and *S. epidermidis* isolates were 100% resistant to Cloxaciline and Kanamycin while the resistance to Penicillin was 100% in *S. aureus* and 33.33% in *S. epidermidis* isolates.

**Keywords:** Goat, Mastitis, Staphylococci, Hemolysin, Antimicrobial susceptibilities, Iran.

### Introduction

Several pathogens can cause mastitis but *Staphylococcus* spp. are the most frequently diagnosed causal microorganisms of intra mammary infection (IMI) in goats. Other pathogens such as *Streptococcus* spp., *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Mannheimia haemolytica*, *Corynebacteria* and fungi can produce IMI in small ruminants (4).

The number of species of the genus *Staphylococcus* is steadily increasing. About 36 species are listed in the 2006 review (10). Some species of this genus cause a variety of diseases by production of a series of enzymes and toxins, invasion of host cells and tissues.

Staphylococcal alpha-hemolysin or alpha-toxin is the most studied and characterized cytotoxin, and is considered a main pathogenicity factor because of its hemolytic, dermonecrotic and neurotoxic effects (9). Additionally, beta-hemolysin is a sphingomyelinase that is highly active against sheep and bovine erythrocytes (13), while delta-hemolysin as well as alpha-hemolysin induce pore formation, perturbing the cell membrane permeability (2).

In reports from different parts of the world the

oxacillin resistance of coagulase-negative staphylococci (CNS) lies between 70% and 80% (23), in a recent study Dar et al., (7) showed that 22.5% of CNS isolates from 750 human subjects were resistant to methicillin. The public health significance of *Staphylococci* isolated from milk and dairy products is important because these products can be a source of toxins and antibiotic-resistant strains for humans (21).

In goat the role of *Staphylococcus aureus* and (CNS) in udder infection was the subject of some recent studies, (3, 11, 20). However, there are few studies on the antimicrobial susceptibilities and exotoxin production by *Staphylococci* isolated from goat mastitis in Iran.

The present study was designed to determine the species, hemolytic ability and antimicrobial susceptibilities of the staphylococci isolated from sub clinical goat mastitis in Shahrekord dairy herds in west central Iran.

### Materials and Methods

#### A- Sample collection

The study covered 20 months from March 2006. A preliminary analysis for presence of sub clinical mastitis was done in 510 native goats from 5 free ranging herds in Shahrekord district, west central Iran,

via CMT test. The numbers of milking goats in herds 1 to 5 were as 180 out of 200, 140 out of 160, 120 out of 160, 40 out of 45 and 30 out of 40 respectively that were followed by CMT test.

Prior to sampling, the udders were inspected and palpated for estimation of pathological alterations. First milk stream followed by visual analysis, to detect macroscopic alteration in milk as an indicator of clinical mastitis too. When such changes were not observed and the CMT was positive, milk samples were aseptically obtained in sterile 10 mL tubes. The ice box containing milk samples were sent to microbiology lab. of veterinary college of Shahrekord university for the isolation of strains. On arrival in the laboratory, aliquots of 0.01 ml of milk were streaked on blood agar with 5% defibrinated ovine blood agar. The incubation was done aerobically at 37 °C for 24-48 h. The presence of more than 3 colonies of a similar morphotype was accepted as positive bacteriological finding (8). Colony morphology, gram staining and catalase test were used to identify bacterial genus and followed by further biochemical tests.

#### B- Identification of Staphylococcus species

The species identification was carried out according to Murray et al., (14). The colonies identified as Staphylococcus were submitted to coagulase test using rabbit plasma (18). The catalase and oxidase tests were followed by examining the susceptibility profile of isolations towards bacitracin. The tests of sucrose, D-mannose, D-manitol, maltose, D-trehalose, raffinose fermentation, urease activity and acetoin production were also assayed. All tests were performed as described by Quinn et al., (18). The isolates were kept frozen at -20 °C in Tryptone soy broth containing 15% (v/v) glycerol, until the hemolytic tests were carried out.

#### C- Essay for hemolytic activity

The hemolytic activity was evaluated by plating Staphylococci strains on triplicate plates of blood agar base supplemented with 5% sheep, bovine and horse blood for alpha, beta and delta-hemolysin assays respectively (18). To remove any possible anti-hemolysin compounds present in the serum, the red blood cells were submitted to washes with sterile saline and resuspended in saline to the original volume of the blood.

Strains were inoculated in the form of streaks on the surface of plates and incubated at 37 °C for 24 and 48 h. The criteria for hemolysin identification were: complete lytic zone (transparent) with blurred edges for alpha-hemolysin on ovine and incomplete (non-transparent) lytic zone, which became complete with sharp edges after overnight incubation at 4 °C on bovine blood agar, for beta-hemolysin. The delta-hemolysin production was determined as complete hemolytic zones on horse blood agar (18, 20).

#### D- Susceptibility testing

For susceptibility testing, isolates were incubated in trypticase soy broth at 37 °C for 24 h and the suspension was adjusted to a turbidity equivalent to a 0.5 McFarland standard. Susceptibility to antimicrobial agents was determined for isolated strains by the disk diffusion method on Mueller-Hinton agar following the National Committee for Clinical Laboratory Standards guidelines (15).

The strains were determined by the two scale system of Bauer-Kirby as sensitive (S) or resistant (R) according to Performance Standards for Antimicrobial Disk Susceptibility Tests (16). The selected 10 antibiotics for antibiogram were Methicillin, Penicillin, Cloxaciline, Gentamicin, Amikacin, Kanamycin, Oxytetracycline, Chloramphenicol, Erythromycin and Norfloxacin.

#### E- Statistical analysis

Different herds IMI and isolated strains were compared using chi square and Fisher's test analysis respectively.

#### Results

Out of 510 CMT tested milk samples, 115 (22.543%) were positive for sub clinical mastitis that were followed by bacteriological examinations. No significant differences were observed between herds IMI and isolated strains ( $P < 0.05$ ). The details are summarized in table 1.

Fourteen strains of *S. aureus* (12.17%) and 9 strains of *S. epidermidis* (7.82%) were isolated. 11 and 6 isolates of *S. aureus* and *S. epidermidis* produced combined form of hemolysins  $\alpha/\beta/d$ , while  $\beta/d$  hemolysins produced by 2 and 3 isolates respectively. Only one isolate of *S. aureus* produced single type d hemolysin.

The antimicrobial susceptibility data for isolates of *S. aureus* and *S. epidermidis* are summarized in table-2. *S. aureus* and *S. epidermidis* isolates were 100% resistant to Cloxaciline and Kanamycin while the resistance to Penicillin was 100% in *S. aureus* and 33.33% in *S. epidermidis* isolates, resistance to methicillin were 28.57% and 22% respectively.

In other hand 100% of isolated species were sensitive to Gentamicin, Amikacin, Oxytetracycline, Chloramphenicol and Norfloxacin.

#### Discussion

As Contreras et al., (4) in a review indicate, subclinical mastitis in small ruminant average 5-30% that covers our result of 22.4%. In that review Staphylococcus spp. appear as the most frequently diagnosed causal microorganisms of IMI in goats. We isolated only 23 strains (22%) of Staphylococci from 115 IMI cases, Since *Contagious agalactiae* is endemic in our area (17) and the disease have intense

Table 1: CMT positives and Staphylococcus species from different goat herds

Herd: no. of milking goats	1: 180	2: 140	3: 120	4: 40	5: 30
no. of CMT positives	40	22	20	20	13
percentage	(22.22%)	(18.33%)	(16.66%)	(50%)	(43.33%)
<i>S. aureus</i>	4 (6.66%)	6 (27.27%)	1 (5%)	2 (10%)	1 (7.69%)
<i>S. epidermidis</i>	4 (6.66%)	0 (0%)	1 (5%)	3 (15%)	1 (5%)

effect on reducing milk production and increasing somatic cell counts (6), our results support this idea that *Contagious agalactiae* should be considered as one of important causes of small ruminant mastitis in endemic areas, where subclinical cases are frequent.

In the present study 14 and 9 isolates of *S. aureus* and *S. epidermidis* were confirmed respectively. IMI caused by *S. aureus* warrant special attention because this bacterium is responsible for both acute clinical and sub clinical mastitis. Radostits et al., (19) asserted that *S. aureus* is well adapted to survive in the udder and usually establishes a mild sub clinical infection over a long duration. Regarding the CNS, our result are in line with Contreras et al., (5) that reported *S. epidermidis* and *S. caprae* among the most prevalent causal microorganisms in IMI infection of goats.

Diseases caused by Staphylococci are the result of a synthesis of several virulence factors including the different hemolysins, which are important for virulence of the *S. aureus* and other species. All of *Staphylococci* isolates demonstrated hemolytic activity either in alone or in combined forms.

A similar study followed in mastitis goat milk in Brazilian dairy herds also showed high level of single or combined hemolysin types produced by *S. aureus* and CNSs (20). In the present study all 9 isolates of *S. epidermidis* produced combined types of hemolysins, there are indications that *S. epidermidis* is more pathogenic for goat mammary glands than the other species belonging to coagulase negative *Staphylococci* (4).

A possible involvement of these hemolysins in different pathological processes was aroused by Butt et al., (2) who reported hemolytic activity in 49% of CNS strains isolated from human chronic muscular disease. Kenny et al., (12) reported that interaction of these toxins in mastitis cases might be lead to clinical signs. The data from this study suggest that goats infected mammary glands are an important source of hemolytic *Staphylococci*.

Resistance of *S. aureus* and *S. epidermidis* to methicillin were 28.57% and 22% respectively, for the former our results are in about with Bochev and Russenova,(1) that reported 20% methicillin resistancy for isolated *S. aureus* strains, but 80% for CNS isolated from goat IMI samples. Our results about methicillin resistancy could be worrisome since the infections caused by methicillin-resistant *Staphylococci* cannot

be effectively treated with beta-lactam antibiotics (7).

The high resistance of *S. aureus* and *S. epidermidis* isolates to Cloxaciline and Kanamycin and *S. aureus* to Penicillin found in this study is also worrisome. More restrictive policies on the use of antibiotics in animals may result in an improvement of the current situation. Other studies show some resistance of CNS to penicillins but a limited occurrence of resistance to other antimicrobial agents (22).

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