

Prevalence of *Staphylococcus aureus* in lactating goats with clinical mastitis and their antibiogram studies

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Abstract. *Staphylococcus aureus* infects animals and humans as normal flora or pathogens. The present study was intended to determine the prevalence of *S. aureus* in clinically infected mastitic goats and to assess the antimicrobial resistance of *S. aureus* isolated from milk from goats with clinical mastitis. From a total of 71 mastitic milk samples 27 isolates were obtained with a recovery rate of 38.03%. The isolates were genotypically confirmed on the basis of 23S rRNA ribotyping. All the isolates were subjected to antibiogram studies using 32 antibiotics belonging to different categories and generations. The analysis of antibiogram obtained revealed that the most effective antibiotics were Gentamicin and Tobramycin (100%) followed by Netillin, Methicillin, Cefalexin, Chloramphenicol, Enrofloxacin, Azithromycin and Nitrofurazone in descending order of their efficacy. All the isolates were resistant to Cefixime and Metronidazole.

Key Words: Antibiogram, goat, clinical mastitis, *Staphylococcus aureus*.

Introduction. Mastitis is a serious concern to both meat and milk producers since the infection can lead to considerable economic losses due to reduction in milk yield, decreased milk quality and treatment costs (Sawant et al 2009; Franca et al 2012). *Staphylococcus aureus* is the most important pathogen of caprine mastitis worldwide (Aras et al 2012) and presence of *S. aureus* in milk has public health significance also, since many of the enterotoxins produced by this organism could result in food poisoning (Fagundes et al 2010; Razi et al 2012).

Antibiotics are used to treat diseases of various animals as well as preservatives for milk (Deveriese et al 1997). The indiscriminate use of antibiotics has led to the development of multiple antibiotic resistances thereby rendering the antibiotic treatment ineffective (Alian et al 2012). In addition *S. aureus* acquires antibiotic resistance with remarkable proficiency (Booth et al 2001). This situation needs new therapeutic agent with novel mechanism of action to circumvent these infections (Benton et al 2004) or the most effective antibiotics among the available lot should be used after testing against the organisms.

The present report adds more to the records of prevalence of *S. aureus* in caprine mastitis and resistance exhibited toward various antibiotics.

Material and Method

A total of 71 milk samples were collected from mastitic goats from Bikaner city (Rajasthan, India) for bacterial isolation. The clinical diagnosis was made on the basis of history, inspection and palpation of the udder and visual examination of the milk samples. The goats with visible abnormalities in milk (watery milk, clots, pus, blood in milk etc.) and physical changes in udders were diagnosed to have clinical mastitis.

Collection of milk samples. Udders and teats of the randomly inspected 71 lactating goats were cleaned with water, dried and then teats were disinfected with 70% ethanol before sampling. The first few streams of milk were discarded to avoid external contamination. About 10 ml milk sample was collected in sterile test tube and transported to laboratory immediately for bacteriological studies.

Isolation and identification of bacteria. The milk samples were inoculated on nutrient agar, blood agar and mannitol salt agar and incubated at 37°C for 24 to 48 hours. The organisms were isolated and identified as methods described by Cowan & Steel (1975) and Quinn et al (1994). Further, confirmation of isolates was done by 23S rRNA ribotyping (Straub et al 1999) using the following species-specific primers, Primer-1: 5'-ACGGAGTTACAAAGGACGAC-3' and Primer 2: 5'AGCTCAGCCTTAACGAGTAC-3'.

Antibiogram. The antibiogram was studied by the method described by Bauer et al (1966) using 32 antibiotics belonging to different generations (Table 1). In brief, the isolates were inoculated in sterile 5 ml nutrient broth and incubated overnight at 37°C. The opacity was adjusted to 0.5 McFarland opacity standards (Quinn et al 1994). The inoculum was swabbed over the Mueller-Hinton agar and plates were allowed to dry for 10 min at 37°C and then antibiotic discs were placed carefully keeping enough space around each disc for proper diffusion of antibiotic. Plates were incubated for 24 h at 37°C. The zone of inhibition of growth of the organism around each disc was measured in millimeters.

Results and Discussion. Goats are important for livelihood of a large number of people in the third world. As goats are important dairy animals in arid areas next to large ruminants, information on mastitis and associated risk factors has vital importance for control interventions (Megersa et al 2010). Bergonier et al (2003) reported that in goats 18% of animals culled or dead for disease reasons experienced mastitis.

Because of pathogenicity for both humans and animals, *S. aureus* has been the main subject of studies on antibiotic resistance (Lyon & Skurray 1987). In the present report the prevalence of *S. aureus* in the milk sample detected was 38.03% (27/71). The results could not be compared due to lack of reports in goats from this area, however, these findings were in accordance with the reports of researchers worldwide, 38% in Austria (Deinhofer & Pernthancer 1995), and 37% in Nigeria (Ameh & Tari 2000). But White & Hinckley (1999) obtained a lesser recovery of 11% of *S. aureus* isolates from mastitic goat milk.

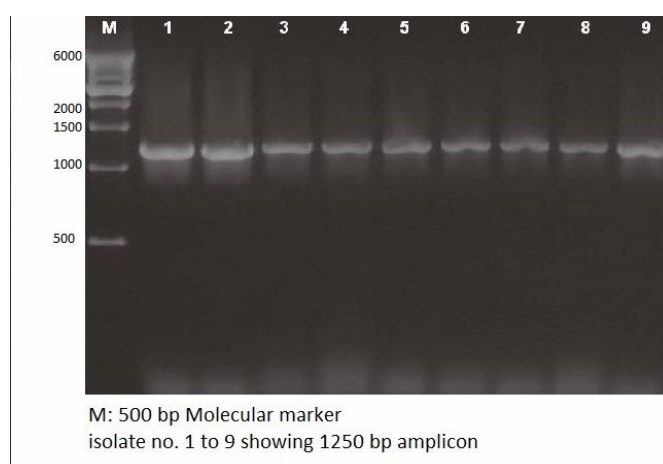


Figure 1. Ribotyping (23 S rRNA) of *Staphylococcus aureus* from lactating goats with clinical mastitis.

All the isolates in the present study produced a species-specific amplicon of 1250 bp (Figure 1) and hence were confirmed as *S. aureus*. Similar genotypic method of *S.*

aureus identification have been used successfully by Sanjiv et al (2008), Upadhyay et al (2010), Khichar et al (2012) and Rathore & Kataria (2012) for the isolates from this study area and Stephan et al (2001), Salasia et al (2004) and Bhanderi et al (2009) for *S. aureus* isolates from elsewhere.

The results of *in vitro* antibiotic susceptibility test of all 27 *S. aureus* isolates to 32 antibiotics are presented in table 1. In the investigation Gentamicin and Tobramycin was found to be the most effective antibiotics followed by Netillin, Methicillin, Cefalexin, Chloramphenicol, Enrofloxacin, Azithromycin, Nitrofurazone and others in descending order of their efficacy. The results are in complete agreement to those of the reports of Mubarak et al (2012) who also found Gentamicin as the most effective (no resistance).

Table 1
Antibiogram pattern of *Staphylococcus aureus* isolates from goats with clinical mastitis

No.	Antibiotic	Percent (%)		
		Sensitive	Intermediate	Resistant
1	Gentamicin	100	-	-
2	Tobramycin	100	-	-
3	Netilin	97.30	-	3.70
4	Methicillin	97.30	-	3.70
5	Chloramphenicol	85.18	14.81	-
6	Cefalexin	85.18	11.11	3.70
7	Azithromycin	81.48	14.81	3.70
8	Enrofloxacin	81.48	-	18.52
9	Nitrofurazone	77.78	-	22.22
10	Rifampicin	74.07	18.52	7.40
11	Ofloxacin	70.37	-	29.63
12	Co-Trimoxazole	70.37	25.92	3.70
13	Cefaclor	66.66	18.51	14.81
14	Amoxyclav	62.96	-	37.03
15	Bacitracin	62.96	37.03	-
16	Sparfloxacin	55.56	14.81	25.93
17	Amoxycillin	37.03	-	62.96
18	Linezolid	37.04	-	62.96
19	Norfloxacin	33.33	25.93	40.74
20	Vancomycin	22.22	-	77.78
21	Azlocillin	14.81	-	85.18
22	Ceftriaxone	14.81	3.70	85.18
23	Cephotaxime	14.81	85.18	3.70
24	Moxifloxacin	14.81	29.63	55.56
25	Levofloxacin	11.11	77.78	11.11
26	Polymyxin B	3.70	-	96.30
27	Cloxacillin	-	66.67	33.33
28	Novobiocin	-	44.44	55.56
29	Neomycin	-	29.63	70.37
30	Oxytetracyclin	-	25.93	74.07
31	Cefixime	-	-	100
32	Metronidazole	-	-	100

Among β -lactam antibiotic group only one of the 27 isolates (3.7%) showed resistance against Methicillin. Azlocillin was found to be least effective, against which 85% of the isolates showed resistance. The resistance towards other antibiotics viz. Ofloxacin, Cloxacillin, Amoxyclav and Amoxycillin was 30%, 33%, 37% and 62% respectively.

Recent studies have demonstrated that the majority of multiple antimicrobial resistant phenotypes are obtained by the acquisition of external genes that may provide resistance to entire class of antimicrobials (White & McDermott 2001).

Sanjiv & Kataria (2006), Upadhyay & Kataria (2009) and Khichar & Kataria (2013) also carried out antibiogram studies from the same study area against cattle and goat mastitis *S. aureus* isolates. They included 11, 20 and 27 similar antibiotics, respectively as in this study. The analysis of results of these four consecutive studies over a period of 7 years revealed that the susceptibility of the organisms against these antibiotics has been greatly reduced.

In our investigation none of the isolates showed susceptibility towards Oxytetracyclin which is similar to the observations of Ebrahimi et al (2007), Salem-Bekhit et al (2010), Virdis et al (2010), Alian et al (2012) for *S. aureus* isolates from different sources. However, contrarily Oxytetracyclin has been detected to be effective against *S. aureus* infections by many researchers (Aires de Sousa et al 2007; Moon et al 2007; Ayedin et al 2009; Ebrahimi & Taheri 2009; Sharma et al 2011).

The resistance by all the isolates towards Cefixime is unusual as this antibiotic is not being used in veterinary practice. The resistance to Cefixime is being reported from this area since 2009 (Upadhyay & Kataria 2009; Rathore & Kataria 2012; Khichar & Kataria 2013). The present observation towards Cefixime resistance has also been reported by Roesch et al (2006) who recorded resistance to Quinupristin-Dalfopristin though these antimicrobial compounds were not allowed for use in animals. It is indicative of the fact that the Cefixime resistant pathogens are being transmitted from animal handlers.

The extensive variability in the antibiogram patterns exhibited by *S. aureus* from different localities and at different time intervals suggests that this organism is changing its response to various antibiotics very frequently, hence, it is imperative to use right antibiotic in control of *S. aureus* infections. Monitoring of antimicrobial susceptibility in pathogenic bacteria and in commensalisms in animals is also recommended by OIE (Acar & Rostel 2001). The increasing incidence of antimicrobial resistant pathogens has severe implications for the future treatments and prevention of infectious diseases in both animals and humans.

Conclusions. The study revealed 38.03% recovery of *S. aureus* in goat mastitis milk samples and the most effective antibiotics against *S. aureus* were Gentamicin and Tobramycin followed by Netillin, Methicillin, Cefalexin, Chloramphenicol, Enrofloxacin, Azithromycin and Nitrofurazone in decreasing order of their efficacy and all the isolates showed complete resistance towards Metronidazole and Cefixime.

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