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Antimicrobial Susceptibility Profiling of *Staphylococcus aureus* Isolates from Bovine Subclinical Mastitis

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Abstract: The present study was undertaken to determine the efficacy of 32 different antibiotics against 38 *S. aureus* isolates obtained from milk samples from cattle with subclinical mastitis. About 6 antibiotics, i.e., Azithromycin, Gentamycin, Chloramphenicol, Tobramycin, Netillin and Neomycin were found 100% effective followed by Methicillin and Linezolid (94.74%), Cotrimoxazole (92.11%), Nitrofurazone (92.10%), Enrofloxacin (89.47%), Bacitracin (84.21%) and Sparfloxacin, Amoxyclav, Cefaclor and Amoxycillin (81.58%). The resistance was 71.05% for Vancomycin and Polymyxin B, 50% for Oxytetracycline, 47.37% for Azlocillin and 36.84% for Novobiocin whereas Cefixime and Metronidazole were completely (100%) ineffective against all the isolates.

Key words: Antibiogram, bovine, subclinical mastitis, *Staphylococcus aureus*, sample

INTRODUCTION

Subclinical mastitis is a major problem affecting dairy animals all over the world causing enormous economical losses to breeders. The subclinical mastitis is also important because it is 15-40 times more prevalent than the clinical form Kelly (2002) is of long duration, difficult to detect and adversely affects the milk quality. *Staphylococcus aureus* is one of the most frequently isolated pathogens from both subclinical mastitis and chronic infections (Singh and Buxi, 1982; Watts, 1988; Suleiman *et al*., 2012). This organism acquires antibiotic resistance with remarkable proficiency (Booth *et al*., 2001) and increased resistance against antibiotics has been reported by many researchers (Gentilini *et al*., 1995; Aarestrup and Jensen, 1998; Myllys *et al*., 1998). Hence, most effective antibiotics are sorely needed to control the infection and if the animals are tested for sub-clinical mastitis status in the herd along with use of suitable effective antibiotics, the prevalence of mastitis can be reduced to a considerable level. Dutta *et al*. (1995) concluded that a farmer can have an appreciable profit if sub-clinical mastitis positive cows are treated in the early or mid-lactation.

The present investigation was carried out to study the efficacy of different antibiotics against *S. aureus* isolated from bovine subclinical mastitis.

MATERIALS AND METHODS

Milk samples, 5-10 mL in quantity were collected during early morning in sterilized test tubes from cattle (H-F crossbred and Rathi, a native breed) with apparently healthy udders from different locations in Bikaner (Rajasthan, India). The samples were immediately taken to the laboratory for further processing.

Somatic cell counting: A 0.1 mL amount from each properly shaken milk sample was withdrawn with pipette and spread evenly on a glass slide in an area of 1 cm^2, dried in air and then few drops of xylene were poured on it and kept for 1 min to dissolve out fat globules. The smear was then fixed with methanol for 2 min, washed with distilled water and stained with Giemsa stain for 30 min. The smear was washed with phosphate buffer saline solution (pH 7.0) and blot dried. The modified technique of leukocyte count described by Prescott and Breed (1910) was followed for total somatic cell count in which 20 randomly selected fields were examined (100). The total numbers of cells counted in 20 fields were multiplied by a common factor 3246.75 to determine the total somatic cell count per ml of milk sample.

Bacterial isolation and identification: The samples were inoculated on nutrient agar, blood agar and mannitol salt

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Table 1: Antibiogram of *S. aureusisolates* from cattle with subclinical mastitis

	Percent		
Antibiotic disc	Sensitive	Intermediate	Resistant
Azithromycin	100.00	-	-
Gentamicin	100.00	-	-
Chloramphenicol	100.00	-	-
Tobramycin	100.00	-	-
Netillin	100.00	-	-
Neomycin	100.00	-	-
Methicillin	94.74	-	5.26
Linezolid	94.74	-	5.26
Cotrimoxazole	92.11	5.26	2.63
Nitrofurazone	92.10	7.90	-
Enrofloxacin	89.47	-	10.53
Bacitracin	84.21	15.79	
Sparfloxacin	81.58	2.63	15.79
Amoxyclav	81.58	-	18.42
Cefaclor	81.58	13.16	5.26
Amoxycillin	81.58	-	18.42
Ofloxacin	78.95	5.26	15.79
Cloxacillin	78.95	18.42	2.63
Levofloxacin	78.94	7.90	13.16
Norfloxacin	76.32	10.53	13.15
Rifampicin	71.05	21.05	7.90
Moxifloxacin	55.26	26.32	18.42
Azlocillin	52.63	-	47.37
Oxytetracycline	50.00	-	50.00
Cefalexin	34.21	55.26	10.53
Cefotaxime	31.58	63.16	5.26
Vancomycin	28.95	-	71.05
PolymyxinB	28.95	-	71.05
Novobiocin	7.90	55.26	36.84
Ceftriaxone	5.26	73.68	21.06
Metronidazole	-	-	100.00
Cefixime	-	-	100.00

agar, incubated aerobically at 37°C for 24 h and the organisms were isolated and identified as described by Cowan and Steel (1974) and Quinn *et al.* (1994). Of the 85 samples 38 isolates of *S. aureus* were obtained which were further confirmed genotypically by r23S rRNA based ribotypining using following sequences for the two primers, Primer 1: 5'-ACGGAGTTACAAAGGACGAC-3' and Primer 2: 5'- AGCTCAGCCTTAACGAGTAC-3'. An amplicon of 1250 bp was obtained with all the isolates confirming them to be *S. aureus* (Fig. 1).

Antibiotic sensitivity test: The method of Bauer *et al.* (1966) was followed to determine the antibiogram against 32 different antibiotics (Table 1). The 18 h old inoculum in nutrient broth with 0.5 McFarland opacity (Quinn *et al.*, 1994) was used to inoculate nutrient agar plates. After drying, the antibiotic discs were placed on the surface, plates were incubated for 24 h at 37°C and the zone of inhibition of growth of the organism around each disc was measured in millimeters.

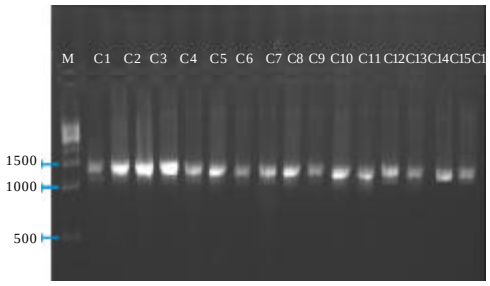

Fig. 1: 23S rRNAribotyping of *S. aureus* isolates obtained from H-F crossbred cattle C1-C16) with subclinical mastitis; M: Molecular weight marker; C1-C16: Isolates from crossbred cattle

RESULTS AND DISCUSSION

The samples which revealed presence of 200×10^3 or more cells/mL along with bacterial growth were included in the present investigation as per the IDF criterion to consider subclinical mastitis. The SCC has been detected to be the most reliable test and closest to the bacteriological results for SCM in dairy cows by Sharma.

The results of antibiogram were interpreted as per the literature supplied by the manufacturer. Accordingly the response of organisms was characterized as sensitive, intermediate and resistant (Table 1). In the present investigation out of the 32 antibiotics, six namely Azithromycin, Gentamycin, Chloramphenicol, Tobramycin, Netillin and Neomycin were found 100% effective against all the isolates. Fourteen of the antibiotics were effective against >75% of the isolates, i.e., Methicillin and Linezolid(94.74%),Cotrimoxazole(92.11%),Nitrofurazone (92.10%), Enrofloxacin (89.47%), Bacitracin (84.21%) and Sparfloxacin, Amoxyclav, Cefaclor and Amoxycillin (81.58%), Ofloxacin and Cloxacillin (78.95%), Levofloxacin (78.94%) and Norfloxacin (76.32%). The remaining 12 antibiotics showed even lesser efficacy. The antibiotics which were not effective against *S. aureus* were cefixime and metronidazole while resistance was 71.05% for Vancomycin and Polymyxin B, 50% for Oxytetracycline, 47.37% for Azlocillin and 36.84% for Novobiocin. The observations are almost similar to those of Suriyasathaporn (2011) who also reported that cloxacillin, gentamicin, oxytetracycline, ampicillin were effective against *S. aureus*causing SCM.

The efficacy of gentamicin towards all the *S. aureus* isolates is in agreement to the observations of many researchers (Lopes *et al.*, 1990; Singh, 2006; Upadhyay and Kataria, 2009; Thaker *et al.*, 2013) who recorded this antibiotic effective against most of the *S. aureus* isolates in their studies. In the present study the susceptibility of *S. aureus* to gentamicin is similar to that

obtained by Ebrahimi and Taheri (2009) who recorded 100% of isolates susceptible to gentamicin but the results for cloxacillin in the present study (78.95%) are opposed to the observations of these researchers where 100% resistance was shown towards this antibiotic.

Sensitivity of *S. aureus* isolates to co-amoxyclav in the present study is comparable to that of Mosaferi *et al.* (2012) who recorded that co-amoxyclav has a moderate effect on *S. aureus* isolated from bovine SCM.

In the present investigation four of the antibiotics viz. methicillin, cloxacillin, amoxycillin and azlocillin belonged to β-lactum antibiotic group and all of these were found effective against most of the isolates. Two of the antibiotics namely methicillin and amoxycillin were found effective against 94.74 and 81.58% of the isolates, respectively whereas cloxacillin andazlocillin were found to be 78.95 and 52.63% effective, respectively. The present findings are in agreement to those of Corti *et al.* (2003) who recorded low resistance of isolates towards penicillin and in contrast to the findings of Pengov and Ceru (2003), Ordonez *et al.* (2004), Kirkan *et al.* (2005), Islam *et al.* (2007), Russi *et al.* (2008), Memon *et al.* (2012) who reported *S. aureus* isolates from bovine clinical and subclinical mastitis showing higher resistance towards penicillin.

Recovery of methicillin resistant strains was very low (5.26%) in the present study which is similar to the findings of Zutic *et al.* (2012) and Khichar (2011) who recovered 5.9 and 3.57% MRSA strains, respectively and in contrast to El-Jakee *et al.* (2010) who recorded higher resistance (60%) by *S. aureus* isolates. Further, 20% resistance towards azithromycin and tobramycin was recorded by El-Jakee *et al.* (2010) which is in contrast to present study where 100% sensitivity to both the antibiotics was recorded.

The finding of inefficacy of cefixime and metronidazole in the present study is in complete agreement to the observations of earlier researchers from this laboratory (Upadhyay and Kataria, 2009; Khichar, 2011; Rathore and Kataria, 2012; Nathawat *et al.*, 2013) who also did not record these antibiotics effective against any of the *S. aureus* isolates recovered from cases of clinical mastitis in cattle and goats and from camel skin wounds.

CONCLUSION

Most of the antibiotics used in the present investigation were effective as the isolates from subclinical mastitis were probably not exposed earlier to these antibiotics. Hence, it is recommended that antimicrobial susceptibility testing should be carried out from time to time so as to use the most effective antibiotics to curb the infection.

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