

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

¹Taruna Bhati, ⁴A.K. Kataria, ²P. Nathawat, ⁴S.K. Sharma, ³N. Mohammed and ¹M. Mathur

¹Department of Animal Husbandry, Government of Rajasthan, India

²Centre for Studies on Wildlife Management and Health,

³Centre for Excellence for Use of Space Based Technology in Animal Science,
College of Veterinary and Animal Science,

⁴Department of Veterinary Microbiology and Biotechnology,
Rajasthan University of Veterinary and Animal Sciences, Bikaner, India

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, Amoxycylav, 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 cm², 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

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

Antibiotic disc	Percent		
	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
Amoxicillin	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 ribotyping 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.

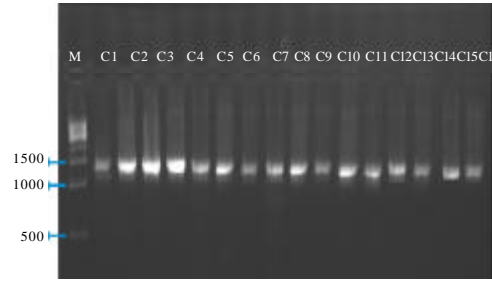


Fig. 1: 23S rRNA ribotyping of *S. aureus* isolates obtained from H-F crossbred cattle C1-C16) with subclinical mastitis; M: Molecular weight marker; C1-C16: Isolates from crossbred cattle

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, Gentamicin, 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 Amoxicillin (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-amoxycylav in the present study is comparable to that of Mosaferi *et al.* (2012) who recorded that co-amoxycylav 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 and azlocillin 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.

REFERENCES

- Aarestrup, F.M. and N.E. Jensen, 1998. Development of penicillin resistance among *Staphylococcus aureus* isolated from bovine mastitis in Denmark and other countries. *Microb. Drug Resist.*, 4: 247-256.
- Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, 45: 493-496.
- Booth, M.C., L.M. Pence, P. Mahasreshti, M. Callegan and M. Gilmore, 2001. Clonal associations among *Staphylococcus aureus* isolates from various sites of infections. *Infect. Immun.*, 69: 345-352.
- Corti, S., D. Sicher, W. Regli and R. Stephan, 2003. Current data on antibiotic resistance of the most important bovine mastitis pathogens in Switzerland. *Schweiz Arch. Tierheilkd*, 145: 571-575.
- Cowan, S.T. and K.J. Steel, 1974. *Cowan and Steel's Manual for the Identification of Medical Bacteria*. Cambridge University Press, Cambridge, UK., pp: 1-229.
- Dutta, G.N., R.K. Saxena and Buragohain, 1995. Economic implications of treatment of lactating cows for sub-clinical mastitis. *Indian Vet. J.*, 72: 420-422.
- Ebrahimi, A. and M.A. Taheri, 2009. Characteristics of staphylococci isolated from clinical and subclinical mastitis cows in Shahrekord, Iran. *Iran. J. Vet. Res.*, 10: 273-277.
- El-Jakee, J., A.S. Nagwa, W.A. Gad El-Said, M.A. Bakry, A.A. Samy, E.A. Khairy and E.A. Elgabry, 2010. Diversity of *Staphylococcus aureus* isolated from human and bovine estimated by PCR-gene analysis. *J. Am. Sci.*, 6: 487-498.
- Gentilini, E., G. Denamiel, L. Tirante, C. Chavez and M.S. Godaly, 1995. Bovine mastitis: β -lactamase production. *Staphylococcus aureus* antibiotic resistance evolution. Proceedings of the 3rd International Mastitis Seminar, Session 2, May 28-June 1, 1995, Tel Aviv, Israel, pp: 84-85.
- Islam, M.I., M.S. Uddin, M.A. Islam, K.H.M.N.H. Nazim, M.T. Rahman and M.M. Alam, 2007. Detection and characterization of coagulase positive among *Staphylococcus aureus* isolated from bovine mastitis in Denmark and other countries. *Microb. Drug Resistance*, 4: 247-256.
- Kelly, A.L., 2002. *Test Methods and Standards*. Academic Press, USA.
- Khichar, V., 2011. Genotypic characterization of *Staphylococcus aureus* from clinical mastitis in cattle in relation to some virulence factors. M.V.Sc. Thesis, Rajasthan University of Veterinary and Animal Sciences, Bikaner, India.

- Kirkan, S., E.O. Goksoy and O. Kaya, 2005. Identification and antimicrobial susceptibility of *Staphylococcus aureus* and coagulase negative *Staphylococci* from bovine mastitis in the Aydin Region of Turkey. Turk. J. Vet. Anim. Sci., 29: 791-796.
- Lopes, C.A., G. Moreno and P.R. Curi, 1990. Antimicrobial susceptibilities of *Staphylococcus aureus* isolated from animal and human sources in Brazil. Br. Vet. J., 146: 50-56.
- Memon, J., J. Kashif, M. Yaqoob, W. Liping, Y. Yang and F. Hongjie, 2012. Molecular characterization and antimicrobial sensitivity of pathogens from sub-clinical and clinical mastitis in eastern China. Pak. Vet. J., 33: 170-174.
- Mosaferi, S., T. Jalili, Z. Ostadi, M. Khakpour and H. Bodaghi, 2012. Sensitivity of *Staphylococcus aureus* isolated from subclinical bovine mastitis to co-amoxiclav in Tabriz dairy herd in 2010. Res. J. Biol. Sci., 7: 165-169.
- Myllys, V., K. Asplund, E. Brofeldt, V. Hirvela-Koski and T. Honkanen-Buzalski *et al.*, 1998. Bovine mastitis in Finland in 1988 and 1995-Changes in prevalence and antimicrobial resistance. Acta Vet. Scand., 39: 119-126.
- Nathawat, P., T. Bhati, S.K. Sharma, N. Mohammed and A.K. Kataria, 2013. Prevalence of *Staphylococcus aureus* in lactating goats with clinical mastitis and their antibiogram studies. Anim. Biol. Anim. Husbandry Int. J. Bioflux Soc., Vol. 5.
- Ordonez, V.V., S. Oaxaca, M.U.A. Fresan, S.L. Bernabe and E. Enrique, 2004. Phenotypic expression of *Staphylococcus aureus* virulence factors isolated from dairy cows with Subclinical mastitis. Int. Soc. Anim. Hyg. Saint Malo, 1: 397-398.
- Pengov, A. and S. Ceru, 2003. Antimicrobial drug susceptibility of *Staphylococcus aureus* strains isolated from bovine and ovine mammary glands. J. Dairy Sci., 86: 3157-3163.
- Prescott, S.C. and R.S. Breed, 1910. The determinants of number of body cells in the milk by a direct method. J. Infect. Dis., 7: 632-640.
- Quinn, P. J., M.E. Carter, B.K. Markey and G.R. Carter, 1994. Clinical Veterinary Microbiology. Wolfe Publishing, London, UK.
- Rathore, P. and A.K. Kataria, 2012. Antimicrobial susceptibility profiling of *Staphylococcus aureus* of camel (*Camelus dromedaries*) skin origin. ABAH Bioflux., 4: 47-52.
- Russi, N.B., C. Bantar and L.F. Calvinho, 2008. Antimicrobial susceptibility of *Staphylococcus aureus* causing bovine mastitis in Argentine dairy herds. Revista Argentina Microbiologia, 40: 116-119.
- Singh, J., 2006. Bacterial determinants of sub-clinical cattle mastitis with special reference to *Staphylococcus aureus*. M.V.Sc. Thesis, Rajasthan Agric. Univ., Bikaner, Rajasthan, India.
- Singh, K.B. and K.K. Buxi, 1982. Studies on etiology, *in vitro* sensitivity and treatment of subclinical mastitis in milch animals. Indian Vet. J., 59: 191-198.
- Suleiman, A.B., J.K.P. Kwaga, V.J. Umoh, E.C. Okolocha and M. Muhammed *et al.*, 2012. Macro-restriction analysis of *Staphylococcus aureus* isolated from subclinical bovine mastitis in Nigeria. Afr. J. Microbiol. Res., 6: 6270-6274.
- Suriyasathaporn, W., 2011. Epidemiology of subclinical mastitis and their antibacterial susceptibility in smallholder dairy farms, Chiang Mai province, Thailand. J. Anim. Vet. Adv., 10: 316-321.
- Thaker, H.C., M.N. Brahmabhatt and J.B. Nayak, 2013. Isolation and identification of *Staphylococcus aureus* from milk and milk products and their drug resistance patterns in Anand, Gujarat. Vet. World, 6: 10-13.
- Upadhyay, A. and A. K. Kataria, 2009. Antibiogram of *Staphylococcus aureus* obtained from clinically mastitic cattle and goats. Vet. Practitioner, 10: 145-147.
- Watts, J.L., 1988. Etiological agents of bovine mastitis. Vet. Microbiol., 16: 41-66.
- Zutic, M., I. Cirkovic, L. Pavlovic, J. Zutic, J. Asanin, O. Radanovic and N. Pavlovic, 2012. Occurrence of methicillin-resistant *Staphylococcus aureus* in milk samples from subclinical mastitis. Afr. J. Microbiol. Res., 6: 5887-5889.