Antibiogram of Bacterial Isolates from Bubaline Subclinical Mastitis

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A B S T R A C T

The present investigation was carried out to study the prevalence of bacterial pathogens responsible for subclinical mastitis in buffaloes in and around Pantnagar in the state of Uttarakhand, India and the antibiogram pattern of the bacterial isolates to selected antibiotics. A total of 109 buffaloes were screened for SCM based on physical examination of udder, California mastitis test (CMT), Somatic cell count (SCC) and Differential cell count (DCC) on quarter's milk samples. The quarter's milk samples showing CMT reaction ≥ 2, SCC ≥ 0.5 million/ml and neutrophils percentage $\geq 40\%$ of total counts were considered for identification of major pathogens involved. Milk samples from affected quarters were subjected to cultural and biochemical examination for isolation and identification of bacterial pathogens. Antibiogram profile of each bacterial isolate to standard antimicrobial discs was determined by agar diffusion method. *Staphylococcus aureus* were the most common isolates of subclinical mastitis in dairy buffaloes, followed by streptococci other than *S. agalactiae*, Coagulase negative staphyllococcus and *S. agalactiae* isolates respectively. The overall antibiogram of the major bacterial isolates revealed the highest sensitivity to enrofloxacin (86.5%), followed by cefotaxime (82.0%) and amoxycillin/clavulanic acid (80.9%). The current work enables selection of proper antibacterials for treatment of mastitis in the study area.

Introduction

Mastitis, inflammation of the mammary gland, is preeminent and expensive disease of dairy animals globally. Pathological alterations in the glandular tissues along with physico-chemical and bacteriological changes in milk are the prominent features of mastitis (Radostits *et al.,* 2007). It could occur in clinical, sub-clinical and chronic form; based on the severity of infection and type of inflammation. However absence of gross inflammation of the gland and major observable abnormalities in milk makes it difficult to detect sub-clinical mastitis. Often ignored, sub-clinical mastitis has a severe economic implications associated with reduced milk production (Viguier *et al.,* 2009). It has been estimated in India that sub-clinical mastitis contributes approximately 60% to the economic setback suffered by dairy sector due to mastitis (Bansal and Gupta, 2009). Both infectious and non-infectious agents could cause mastitis.

Bacteria, yeasts, mycoplasma and various other microbes have been implicated as mastitis causing pathogens (Bradley, 2002). However, intramammary bacterial infection is the major cause of mastitis in dairy animals (Zhao and Lacasse, 2008). Proper microbial diagnosis, prevalence study in the herd and appropriate selection of antimicrobial agents based on antibiotic sensitivity are significant for successful and efficient management of sub-clinical mastitis. Keeping the above facts in mind, the present investigation was carried out to study the incidence of bacterial pathogens responsible for subclinical mastitis in buffaloes and the antibiogram pattern of the isolates to selected antibiotics.

Materials and Methods

The study was conducted on lactating buffaloes of Murrah breed from both organized and unorganized dairy farms in and around Pantnagar located in the Terai region of Himalayas in the state of Uttarakhand, India. A total of 109 buffaloes were screened for SCM on the basis of physical examination of udder, California mastitis test (CMT), Somatic cell count (SCC) and Differential cell count (DCC) on quarter's milk samples. The quarter's milk samples showing CMT reaction $\geq$ 2, SCC $\geq$ 0.5 million/ml and neutrophils percentage $\geq$ 40% of total counts were considered for identification of bacterial pathogens involved. The quarter's milk samples positive on basis of CMT, SCC, DCC and confirmed positive for intramammary infection were classified into subclinical mastitis as per International Dairy Federation criteria for subclinical mastitis while those with inflammatory response but negative on culture examination were classified into non-specific mastitis. Milk samples from the affected quarters were subjected to cultural and biochemical examination for bacterial isolation and identification (Quinn *et al.,* 2004). Antibiogram profile of each bacterial

isolate to standard antimicrobial discs was determined by agar diffusion method using 21 commercially available anti-bacterial sensitivity discs (Bauer *et al.,* 1966). Results of anti-microbial sensitivity were interpreted as sensitive, intermediate or resistant and are expressed as percentage sensitivity to each antibacterial agent.

Results and Discussion

A total of 426 quarters from 109 lactating buffaloes have been investigated of which, 117 quarters from 41 buffaloes were screened positive SCM. Out of these 117 quarter's milk samples screened positive to CMT, SCC and DCC, 83 milk samples from 41 buffaloes were found culturally positive for either single or mixed bacterial infection and classified into subclinical mastitis while 34 samples showed no growth on culture medium and classified into non-specific mastitis. A total of 113 isolates were identified based upon culture and biochemical characteristics out of which *Staphylococcus aureus* were the most common isolates of subclinical mastitis in dairy buffaloes, followed by streptococci other than *S. agalactiae*, Coagulase negative staphyllococus and *S. agalactiae* isolates respectively. A relative distribution of bacterial pathogens of subclinical mastitis in dairy buffaloes in Pantnagar is depicted in Fig. 1.

Staphylococci were found to be the foremost etiological agent of disease and its high prevalence has been reported by several workers in India (Ali *et al.,* 2015 and Sharma *et al.,* 2018) and abroad (Hamed and Ziatoun, 2014; Elsayed *et al.,* 2015). *Streptococci* were the second most prevalent causative agent of bubaline mastitis in and around Pantnagar. This is in close agreement with the findings of Khan *et al.,* (2004) and Sharma and Sindhu (2007). On the contrary, Kumar *et al.,* (2007)

and Jhambh *et al.,* (2017) reported *Streptococci* to be more prevalent than *Staphylococci*. In the present study, 8.41% of cases sub clinical bubaline mastitis was due to *E. coli* infection. Sharma and Sindhu (2007) also recorded comparable occurrence of coliform mastitis in buffaloes whereas Awandkar *et al.,* (2009) have reported higher incidence of *E. coli* infections (40%) in bovine mastitis. Coliform mastitis is indicative of poor hygienic conditions (Sumathi *et al.,* 2008). *Staphylococcus aureus* and *Streptococcus agalactiae* are the most frequent contagious pathogens of bovine mammary gland. *S. aureus* is chief pathogen responsible for SCM in dairy animals (Radostits *et al.,* 2007) while *S. agalactiae* is a noteworthy cause of chronic mastitis where control measures for contagious mastitis have not been properly implemented (Keefe, 1997). Thus, the present investigation reveals the preponderance of the contagious form of subclinical mastitis at the farm that needs to be restricted with apt preventive measures to check further spread. Whereas, a lower prevalence of SCM due to *E. coli* and other environmental pathogens (Radostits *et al.,* 2007) is suggestive of the improved sanitation and hygienic practices at the farm.

Table.1 Overall Antibiogram profile of major bacterial pathogens of subclinical mastitis in dairy buffaloes in and around Pantnagar

Antimicrobial agent	*Staph. aureus*	CoNS	*Strep. agalactiae*	Other streptococci	Overall
	(26)	(23)	(15)	(25)	(89)
Amoxycillin	9 (34.6%)	5 (21.7%)	8 (53.3%)	12 (48.0%)	34 (38.2%)
Amoxycillin/Clavulanic acid	17 (65.3%)	19 (82.6%)	15 (100%)	21 (84.0%)	72 (80.9%)
Amoxycillin/Sulbactum	12 (46.1%)	10 (43.4%)	10 (66.6%)	16 (64.0%)	48 (53.9%)
Ampicillin	9 (34.6%)	6 (26.1%)	6 (40.0%)	10 (40.0%)	31 (34.8%)
Ampicillin/Cloxacillin	11 (42.3%)	6 (26.1%)	9 (60.0%)	15 (60.0%)	41 (46.1%)
Ampicillin/Sulbactum	17 (65.3%)	11 (47.8%)	11 (73.3%)	18 (72.0%)	57 (64.0%)
Cefotaxime	19 (73.1%)	22 (95.6%)	11 (73.3%)	21 (84.0%)	73 (82.0%)
Ceftriaxone	18 (69.2%)	18 (78.2%)	12 (80.0%)	22 (88.0%)	70 (78.6%)
Clindamycin	13 (50.0%)	22 (95.6%)	12 (80.0%)	18 (72.0%)	65 (73.0%)
Cloxacillin	10 (38.4%)	11 (47.8%)	12 (80.0%)	19 (76.0%)	52 (58.4%)
Enrofloxacin	22 (84.6%)	23 (100%)	14 (93.3%)	18 (72.0%)	77 (86.5%)
Erythromycin	15 (57.7%)	22 (95.6%)	12 (80.0%)	15 (60.0%)	64 (71.9%)
Gentamicin	16 (61.5%)	14 (60.8%)	10 (66.6%)	15 (60.0%)	55 (61.7%)
Lincomycin	13 (50.0%)	20 (86.8%)	12 (80.0%)	19 (76.0%)	64 (71.9%)
Methicillin	13 (50.0%)	6 (26.1%)	10 (66.6%)	22 (88.0%)	51 (57.3%)
Neomycin	20 (76.8%)	18 (78.2%)	13 (86.6%)	18 (72.0%)	69 (77.5%)
Ofloxacin	10 (38.4%)	13 (56.5%)	9 (60.0%)	14 (56.0%)	43 (48.3%)
Penicillin G	5 (19.2%)	5 (21.7%)	6 (40.0%)	10 (40.0%)	26 (29.2%)
Rifampicin	17 (65.3%)	17 (73.9%)	12 (80.0%)	21 (84.0%)	67 (75.2%)
Streptomycin	16 (61.5%)	14 (60.8%)	14 (93.3%)	19 (76.0%)	63 (70.7%)
Tetracycline	14 (53.8%)	14 (60.8%)	10 (66.6%)	15 (60.0%)	53 (59.5%)

Fig.1 Relative distribution of bacterial pathogens of subclinical mastitis in dairy buffaloes
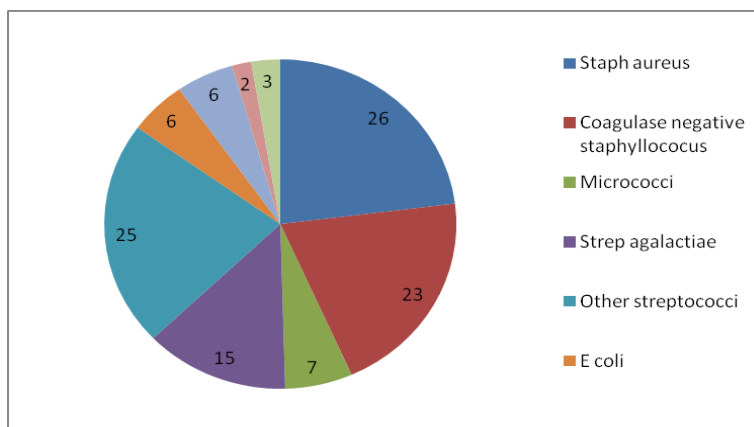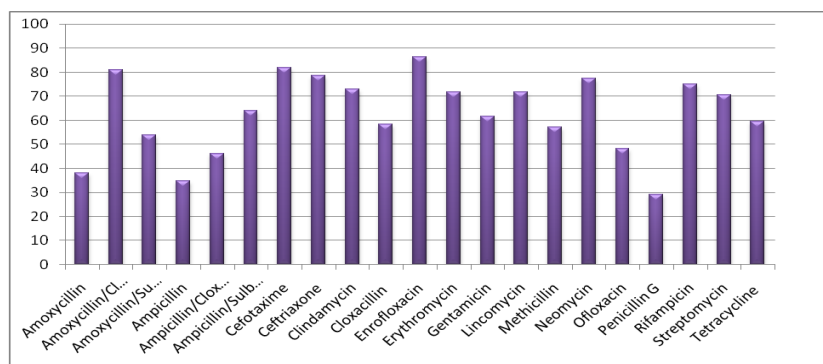

Fig.2 Antibiogram profile of major bacterial pathogens of subclinical mastitis in dairy buffaloes in and around Pantnagar

Percent antimicrobial sensitivity of major bacterial isolates of mastitic milk in and around Pantnagar is depicted in table 1 and figure 2. The antibiogram of the *Staphylococcus aureus* isolates revealed the highest sensitivity to enrofloxacin followed by neomycin, cefotaxime, ceftriaxone, amoxycillin/clavulanic acid, ampicillin/ sulbactam, rifampicin, gentamicin, streptomycin, erythromycin, tetracycline, lincomycin, clindamycin, methicillin, amoxycillin/ sulbactam, ampicillin/ cloxacillin, cloxacillin, ofloxacin and least sensitivity to amoxycillin, ampicillin and penicillin G.

Enrofloxacin was effective against 100% isolates of Coagulase negative *Staphylococci.*

Amoxycillin/clavulanic acid (100%) followed by Enrofloxacin and Rifampicin were highly effective against *Streptococcus agalactiae* while against other *Streptococci* species other than *Streptococcus agalactiae*, methicillin and ceftriaxone were most effective followed by amoxycillin/clavulanic acid and cefotaxime.

The overall antibiogram of the major bacterial isolates revealed the highest sensitivity to enrofloxacin (86.5%), followed by cefotaxime (82.0%), amoxycillin/clavulanic acid (80.9%), ceftriaxone (78.6%), neomycin (77.5%), rifampicin (75.2%), clindamycin (73.0%), lincomycin (71.9%), erythromycin (71.9%), streptomycin (70.7%), ampicillin/sulbactam (64.0%), gentamicin (61.7%), cloxacillin (58.4%), methicillin (57.3%), amoxycillin/

sulbactam (53.9%), ofloxacin (48.3%), ampicillin/cloxacillin (46.1%) and least sensitivity to amoxycillin (38.2%), ampicillin (34.8%), and penicillin G (29.2%).

Tripathi (2015) studied the antimicrobial sensitivity pattern of bacterial isolates of subclinical mastitis in cows at the same farm which also showed the highest sensitivity to enrofloxacin and cefotaxime. Somewhat similar antibiogram pattern of bacterial isolates has been recorded by Bhanot *et al.,* (2012) and Ali *et al.,* (2015). Poor sensitivity to penicillin G and amoxycillin might be due to the production of β-lactamase enzyme by resistant strains of bacteria owing to their frequent use at the farm for mastitis control. On the other hand, higher sensitivity to enrofloxacin, cefotaxime, amoxycillin/ clavulanic acid, might be explained on the basis of their less frequent use at the farm. *In vitro* antimicrobial susceptibility is considered as a prerequisite for treatment. However, *in vitro* activity does not guarantee *in vivo* efficacy as pharmacokinetics of the antimicrobial substance greatly affects its suitability for mastitis treatment (Pyöräla, 2009).

The variation in the present study in the sensitivity pattern could be credited to the variation in sensitivity of different isolates in different geographical locations and resistance to commonly used antibacterials. Further, indiscriminate use of these drugs contribute to the increased resistance of different bacterial strains to commonly used antibacterials such as Ceftriaxone, Cefotaxime, Ceftriaxone/ Salbactum, Amoxycillin/ Sulbactam and streptomycin.

Sahoo *et al.,* (2009) was of the opinion that antibacterial sensitivity test of different antibacterials varies widely from low to high sensitivity could be attributed to their prolonged and injudicious usage under field conditions. The current work enables selection of proper antibacterials for treatment of sub-clinical mastitis in the study area. Further, this study warrants usage of drugs at proper dosages and schedule to prevent further antibacterial resistance to different bacteria.

Acknowledgements

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