Research Article

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ANTIMICROBIAL SENSITIVITY PATTERN OBSERVED IN MICROBES ASSOCIATED WITH BOVINE MASTITIS

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ABSTRACT

A total of 9328 quarter milk samples from 2755 cows and buffaloes received at the college central laboratory during the year July 2015 to June 2016 were evaluated for isolation and *in vitro* antimicrobial sensitivity pattern. A total of 8010 bacterial isolates from 6005(64.4%) (54.5%) culture positive milk samples were obtained. Of the total isolates, higher prevalence of *Staphylococcus* spp. (53.9%) followed by *Streptococcus* spp. (29.7%), *E. coli.* (10.2%), *Diplococcus* spp.(3.2%), *C. pyogenes* (1.0%), *Klebsiella* spp.(0.8%), *Pseudomonas aeruginosa* (0.5%), *Bacillus* spp. (0.5%) and *Pasteurella multocida* (0.1%) were found. About 33.4% culture positive samples revealed mixed infections of which the most prevalent combination was *Staphylococcus* spp. and *Streptococcus* spp. The most resistant antibiotics against Gram-positive bacteria were penicillin (87.3%) and streptomycin (77.9%). Similarly, penicillin was found to be the most resistant (94%) antibiotic against Gram-negative isolates. Individually, *E. coli* was found to be more sensitive for chloramphenicol, gentamicin, amikacin and enrofloxacin; *Pseudomonas aeruginosa* was more sensitive to chloramphenicol, enrofloxacin, gentamicin and amikacin; *Klebsiella* spp. was more sensitive to chloramphenicol and gentamicin and; *Pasteurella multocida* revealed higher sensitivity towards gentamicin, ceftriaxone, neomycin, amikacin, oxytetracycline and cefoperazone.

Key words: Antimicrobials, Bovine, Buffalo, Cattle, Mastitis, Sensitivity

Mastitis is considered one of the important diseases of the dairy animals because it causes direct economic losses in terms of reduced milk yield and quality, discard of milk due to bacterial and antibiotic contamination, culling of animals and treatment cost. The economic losses due to mastitis have been estimated to be Rs. 7165.51crores per year in India (Bansal and Gupta, 2009). A plethora of pathogens such as *Staphyolococcus* spp. *Streptococcus* spp., *E. coli, Klebsiella* spp. and *Corynebacterium* spp. are associated with mastitis. Antimicrobials are used extensively in the dairy industry for containment of these pathogens from infected udder which in turn also increases the antibiotic residues in milk. The indiscriminate use of antibiotics may also lead to increased bacterial resistance against antimicrobials. One of the important reasons for treatment failure is indiscriminate use of antibiotics without pre-treatment antimicrobial sensitivity testing (WHO, 2000).The occurrence of disease is an outcome of interplay between three major factors: infectious agents, host resistance, and environmental factors (Gera and Guha, 2011). Multi-drug resistant bacteria are a persistent problem in modern health care, food safety and animal health. There is a need for new antimicrobials to replace over used conventional antibiotics (Becker *et al.*, 2016). Therefore, a bacteriological diagnosis, prevalence study on mastitis and proper selection of antibiotic based on antibiotic sensitivity are critical for rational and effective control of mastitis. Strategies involving prudent use of antibiotics for treatment encompass identification of the pathogen and determining the susceptibility/resistance of the pathogen to assess the most appropriate antibiotic to use for treatment. The aim of the present study was to identify bacteria isolated from milk samples submitted from bovine mastitis cases and to evaluate their antimicrobial sensitivity pattern.

MATERIALS AND METHODS

Collection of milk samples: Atotalof9328 quarter milk samples from 2755 dairy cows (n = 913) and buffaloes (n = 1842) from different districts of Haryana during the year July 2015 to June 2016 were screened for prevalence and antimicrobial sensitivity pattern of mastitis pathogens. Animals were categorized as having clinical mastitis having gross abnormality in the udder and milk, whereas in sub-clinical mastitis, no visible change was observed in udder parenchyma and milk.

Bacterial isolation: Quarter milk samples were analyzed microbiologically as per method of Carter *et al*. (1995). In brief, about 0.01 ml of milk sample was inoculated on blood agar enriched with 5% sterile sheep blood and MacConkey lactose agar following aseptic procedures. After inoculation, the plates were incubated at 37°C overnight in incubator. Bacterial isolates were identified by their colony morphology and Gram's staining as described by Carter *et al.* (1995).

Antimicrobial sensitivity testing: Sensitivity of isolates was determined by the disc diffusion method as described by Bauer *et al.* (1966) and the interpretation was made as per the zone size interpretation chart provided by the manufacturer of discs(M/s HiMedia Laboratories Ltd., Mumbai, India). All the-bacterial isolated were analyzed for thirteen different antimicrobials discs, namely, penicillin, streptomycin, oxytetracycline, chloramphenicol, ampicillin, neomycin, cloxacillin, enrofloxacin, gentamicin, amikacin, amoxycillin, ceftriaxone and cefoperazone.

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Table 1
Isolation of bacterial pathogens from clinical and sub-clinical mastitis cows and buffaloes

	Clinical mastitis		Sub-clinical mastitis		Total
	Cow (405)	Buffalo (563)	Cow (508)	Buffalo (1279)	2755
Total quarters	1620	2252	2032	5116	11020
Quarters examined	1404	1841	1813	4270	9328
Culture positive	862	1243	1107	2793	6005
Culture negative	542	598	706	1477	3323
Total isolates	1036	1735	1363	3876	8010
Staphylococcus spp.	615	833	818	2051	4317
Streptococci	229	581	360	1212	2382
E. coli	121	181	117	400	819
C. pyogenes	24	30	6	19	79
Bacillus spp.	5	7	2	21	35
Klebsiella spp.	7	12	11	33	63
P. aeruginosa	7	14	3	16	40
Diplococci	28	66	46	117	257
Pasteurella spp.	-	11	-	7	7
Mixed infections	174*	492**	256***	1083****	2005

* *Staphylococcus* spp. +*Streptococcus* spp. (107), *Staphylococcus* spp.+*E. coli* (38), *Staphylococcus* spp.+ *C. pyogenes* (2), *Streptococci.* + *E. coli* (2), *Staphylococcus* spp.+ *Bacillus* spp. (1), *E. coli+Pseudomonas aeruginosa* (1), *Streptococcus* spp.+ *Bacillus* spp. (4), *Staphylococcus* spp.+*Diplococcus* spp. (8), *Staphylococcus spp+Klebsiella* spp. (5), *E. coli+Diplococcus* spp. (1), *Streptococci+Diplococcus* spp. (3)

** *Staphylococcus* spp. +*Streptococcus* spp.(358), *Streptococcus* spp.+ *C. pyogenes* (8), *Staphylococcus* spp.+*E. coli* (63), *Streptococcus* spp. + *E. coli* (6), *E. coli+Peudomonas aeruginosa* (4), *Staphylococcus* spp. +*Diplococcus* spp. (20), *Streptococcus* spp.+ *Diplococcus* spp. (18), *Staphylococcus* spp. + *C. pyogenes* (2), *Staphylococcus* spp.+*Klebsiella* spp. (7), *Diplococcus spp.+E. coli* (5), *Streptococcus* spp.+ *Klebsiella* spp. (1)

*** *Staphylococcus* spp. +*Streptococcus* spp.(170), *Staphylococcus spp.+E. coli* (52), *Staphylococcus* spp. +*Diplococcus* spp. (28), *Staphylococcus* spp.+*Peudomonasaeruginosa* (2), *Staphylococcus spp.+Klebsiella spp.* (3), *E. coli+ Pseudomonasaeruginosa* (1)

**** *Staphylococcus* spp. +*Streptococcus* spp.(833), *Staphylococcus* spp.+*E.coli* (156),*Streptococcus* spp.+*Diplococcus* spp.(20), *Streptococcus* spp.+ *E. coli* (7), *Streptococcus* spp.+ *C. pyogenes(1), E. coli+Pseudomonas aeruginosa* (2), *Staphylococcus* spp. + *C. pyogenes* (5), *Staphylococcus* spp. +*Klebsiella* spp. (13), *Staphylococcus* spp. +*Bacillus* spp.(4), *Staphylococcus spp.+Diplococcus* spp. (31), *E. coli+Diplococcus* spp. (4), *Staphylococcus* spp. + *Pseudomonas aeruginosa* (7)

RESULTS AND DISCUSSION

In the present study, a total of 9328quarter milk samples (912 cows and 1842 buffaloes; 34.5% and 65.5% respectively) were subjected to bacteriological examination (Table 1). Overall, prevalence of sub-clinical mastitis (64.11%)was found to be similar as compared with clinical mastitis (64.9%) based on the quarters examined. Clinical mastitis can easily be diagnosed as there is gross abnormality in the udder and milk, whereas in sub-clinical mastitis, udder tissue and milk apparently looks normal, hence, most of times it goes unnoticed and contributes as a reservoir of microorganisms leading to spread of infection to other herd mates (Sharma *et al.*, 2015).The occurrence of clinical mastitis would be relative to prevalence of sub-clinical mastitis, as an existing sub-clinical phase of intra-mammary infection predisposes the former causing more economic losses due to decreased milk production, treatment costs and culling (Sindhu *et al.*, 2009; Sharma *et al.*, 2015). Therefore, timely detection of sub-clinical mastitis is important to initiate proper treatment, control and preventive measures. Both in cows and buffaloes, maximum cases were of chronic nature (49.9% and 57.8%, respectively) followed by subacute (44.2% and 38.4%, respectively) and acute (5.9% and 3.9%, respectively) cases. In both species, majority(70-80%) of the cases were reported during the 1st four parities and during the 1st four months of lactation.

Pattern of isolation of organisms from sub-clinically and clinically infected quarters was similar (Table 1). As many as 6005 (64.4%) samples were found positive for bacteriology. A total of 8010 isolates were obtained from infected quarters of cows and buffaloes. Of these, 53.9% were *Staphylococcus* spp., 29.7% were *Streptococcus* spp., 10.2% were *E. coli,* 3.2% were *Diplococcus* spp., 1.0% were *C. pyogenes,* 0.8% were *Klebsiella* spp., 0.5% were *Pseudomonas aeruginosa,* 0.4% were *Bacillus* spp. and 0.1% were *Pasteurella multocida*. Out of 6005 culturally positive samples, 33.4% revealed mixed infections in different combinations. Most prevalent combination of the isolates was *Staphylococcus* spp. + *Streptococcus* spp. followed by other combinations. Earlier studies also revealed that *Staphylococcus* spp. was the major pathogen causing mastitis in dairy cattle in India (Bhalerao *et al.,* 2000; Sharma and Prasad, 2002; Sharma *et al.*, 2007; Verma *et al.*, 2018) and other parts of the world (Hawari and Dabas, 2008; Nickerson, 2009). Distribution of pathogens in mastitis changes over time, therefore, bacteriological examination at herd level must be taken regularly to monitor udder health. The higher incidence of *Staphylococcus* spp. indicates unhygienic milking practices as this pathogen mainly spread during milking via milker's hands or by milking machines (Verma *et al.*, 2018). The bovine mammary gland can be a significant reservoir of enterotoxigenic strains of *Staphylococcus aureus* whereas prevalence of *E. coli* is an indication of poor hygienic practices in dairy as these organisms originate from the cow's environment and infect the udder through the teat canal. Contamination of end of the teat is a major predisposing factor in development of environmental mastitis (Bradley, 2002). *Streptococcus* spp. accounted for second most prevalent causative agent of mastitis in this study. The present results are in accordance with the previous studies reported by Khan *et al.* (2004) and Sharma and Sindhu (2007). On contrary, Kumar *et al.* (2009) reported *Streptococcus* spp.to be more prevalent than *Staphylococcus* spp. In the present study, 10.2% mastitis cases were due to *E. coli*

Table 2
***In vitro* antimicrobial sensitivity pattern (%) of bacterial isolates from mastitis cows**

Antimicrobial drug	*Staphylococcus* spp. (1504)	*Streptococcus* spp. (664)	*C. pyogenes* (28)	*Diplococcus* spp. (65)	*E. coli* (264)
Penicillin	16.2	17.3	39.3	15.4	0.75
Streptomycin	30.4	18.1	60.7	6.2	28.0
Oxytetracycline	30.1	25.3	50.0	26.2	14.4
Chloramphenicol	70.9	62.8	78.6	76.9	75.4
Ampicillin	44.9	44.9	60.7	53.8	8.7
Neomycin	39.8	27.4	60.7	12.3	37.5
Cloxacillin	48.9	38.0	60.7	29.2	3.0
Enrofloxacin	46.0	40.7	78.6	10.8	49.6
Gentamicin	56.1	49.4	75.0	30.8	61.7
Amikacin	44.7	28.6	67.9	12.3	37.3
Amoxycillin	35.6	37.8	53.6	47.7	7.6
Ceftriaxone	47.7	49.2	75.0	53.8	45.5
Cefoperazone	47.8	40.2	71.4	47.7	20.1

Table 3
***In vitro* antimicrobial sensitivity pattern (%) of bacterial isolates from mastitis buffaloes**

Antimicrobial drug	*Staphylococcus* spp. (2904)	*Streptococcus* spp. (1889)	*C. pyogenes* (62)	*Diplococcus* spp. (198)	*Bacillus* spp. (30)	*E.coli* (657)	*Pseudomonas aeruginosa* (32)	*Klebsiella* spp (57)	*Pasteurella multocida* (18)
Penicillin	12.7	17.6	37.1	26.8	13.3	0.6	0.0	0.0	05.55
Streptomycin	26.4	22.1	40.3	30.3	40.0	37.0	65.6	43.9	38.9
Oxytetracycline	31.2	28.1	45.2	49.0	43.3	35.8	46.9	33.3	66.7
Chloramphenicol	68.8	67.1	83.9	79.3	56.7	75.6	81.3	65.0	66.7
Ampicillin	36.7	43.4	54.8	79.3	50.0	18.4	28.1	1.8	33.3
Neomycin	33.9	33.6	48.4	41.4	40.0	42.3	68.8	57.9	77.8
Cloxacillin	41.7	33.5	54.8	42.4	43.3	1.5	0.0	0.0	38.4
Enrofloxacin	42.6	42.0	71.0	42.4	70.0	61.5	75.0	78.9	66.7
Gentamicin	54.1	49.6	74.2	54.0	56.7	64.2	71.9	64.9	94.4
Amikacin	46.6	37.2	58.1	33.3	56.7	57.4	71.9	59.6	72.2
Amoxycillin	36.2	42.6	62.9	66.7	50.0	14.9	25.0	8.8	27.8
Ceftriaxone	42.7	44.1	74.2	65.7	53.3	51.3	59.4	50.9	77.8
Cefoperazone	41.2	41.8	72.6	62.1	53.3	46.4	68.8	43.9	66.7

infection. Sharma and Sindhu (2007) also recorded 11.8% occurrence of coliform mastitis in infected buffaloes. On the other hand, Awandkar *et al.* (2009) reported higher incidence of E. coli infections (40.0%) in bovine mastitis. The high percentage of mastitis caused by *E.coli* indicates contamination from soil and faecal matter. Verma *et al.* (2018) observed that major prevalent pathogens isolated from mastitis were *Staphylococcus* spp. (42.6%), *E. coli* (21.3%), *Streptococcus* spp. (6.4), *Proteus* spp. (8.5%), *Candida* spp. (2.9%) and mixed infection (18.3%).

Early treatment of mastitis with effective antibiotics significantly decreases the severity of mastitis, economic loss and development of antimicrobial resistance. So all isolates obtained from cows and buffaloes samples were subjected to invitro antimicrobial sensitivity testing (Table 2 and 3). In case of buffaloes while considering overall pattern, all Gram-positive bacteria showed high sensitivity towards chloramphenicol (79.3 to 83.9%) and ceftriaxone (65.6 to 74.2%). Almost similar sensitivity pattern was observed in cows except that cow isolates also showed high sensitivity towards gentamicin (49.4 to 75.0%). *E.coli* isolates from both cows and buffaloes revealed high sensitivity towards chloramphenicol, gentamicin, amikacin and enrofloxacin. Pseudomonas aeruginosa isolates were highly sensitive to chloramphenicol, enrofloxacin, gentamicin and amikacin, whereas *Klebsiella* spp.depicted high sensitivity towards chloramphenicol and gentamicin. *Pasteurella multocida* isolates revealed high sensitivity to gentamicin, ceftriaxone, neomycin, amikacin, oxytetracycline and cefoperazone (Table 2 and 3). Whereas, Verma *et al.* (2018) foundthat gentamicin (66.0%) was the most effective antibiotic followed by enrofloxacin (63.8%), cefotaxime+clavulanic acid (52.1%), amoxicillin+sulbactum (42.6%), ciprofloxacin (41.5%), colistin (41.5%), chloramphenicol (39.4%) and ampicillin+sulbactum (38.3%). Least effective antimicrobials were oxytetracycline (22.3%) and streptomycin (25.5%), whereas, maximum resistance drug were found amoxicillin+clavulanic acid (8.5%) and ampicillin+cloxacillin (8.5%). Similar or different antibiogram patterns were reported by other workers (Sumathi *et al.,* 2008, Awandkar *et al.,* 2009 and Sharma *et al.*, 2015). It is not possible to compare antibiogram patterns obtained in the present study with that of previous similar studies. It may be attributed to different factors associated with animal, herd, management, geographical conditions, mastitis control measures and laboratory techniques that may greatly influence the antimicrobial sensitivity results. However, the findings of these studies indicated that indiscriminate and frequent use of antibiotics in animals could be the reason for their ineffectiveness against mastitis causing bacteria (Awandkar *et al*., 2009).

The data presented in this study may provide a good estimate of antimicrobial susceptibility of mastitis pathogens encountered in field conditions. Moreover, due to lack of prophylactic agents, chemotherapy continues to play a major role in therapeutic management of the disease. For success of the treatment, the antibiotic sensitivity tests play a major role in the judicial use of antibiotics in milk producing animals. Improper dosing, indiscriminate and wide spread use of antibiotics and resulting selection pressure lead to rise in antibiotic resistance (Sharma *et al.,* 2015). These resistant bacteria may transfer antibiotic resistance to sensitive population of other bacteria through chromosomal changes, exchange of genetic material via plasmids and transposons (Sharma *et al.,* 2015). It could be concluded from the present study that rational use of antibiotic and regular antibiogram surveillance should be made part of the mastitis control approach.

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