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Occurrence of Clinical Mastitis in Cattle with Emphasis on Antibiogram of Staphylococci

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ABSTRACT

The present study was conducted on clinical cases of mastitis in cattle where isolation, identification of udder pathogens and antimicrobial susceptibility of isolated staphylococci was carried out. A total of 565 quarters milk samples of 142 cattle were investigated and among these 394 quarters were found to be infected. Occurrence of mastitis was found to be highest during third and fourth parity and up to first month of post-partum. The highest frequency of *Staphylococcus* spp. (60.04%) was observed followed by *Streptococci* spp. (24.02%), *E. coli* (10.26%) and other bacteria (5.68%). Antimicrobial susceptibility testing revealed that highest resistance of *Staphylococcus* spp. was towards penicillins; cloxacillin, oxacillin, penicillin, ampicillin, amoxicillin and oxytetracycline, while sensitivity was higher towards methicillin, cefuroxime, lincomycin and gentamicin. High occurrence of mastitis needs timely and appropriate strategies to control the spread of this disease. Antimicrobial susceptibility testing should be performed for selection of effective antimicrobial therapy and the judicious use of antibiotics should be done to reduce resistance against antimicrobials in pathogens.

Introduction

India ranked first in the world for production of milk, accounting 176.3 million tonnes with 375 grams per day per-capita availability during 2017-18 (NDDB 2019). To utilize the full production potential of dairy animals and to increase the quantity as well as quality of milk there is need of proper diagnosis and efforts are desired to decrease the occurrence of diseases which are responsible for low milk output from animals. Mastitis is great challenge to dairy sector as it is difficult to manage and has global negative impact on economy. *Staphylococci* spp. is a major contagious pathogen leading to intramammary infections in bovines, which are difficult to cure (Feng *et al.,* 2016). Biofilm formation by *Staphylococci* spp. may lead to emergence of antibiotic resistant strains. Appearance of resistant pathogens against a particular antibiotic in a specific region may be attributed by its frequent and long-term use (Kumar *et al.,* 2010). Continuously varying pattern of antimicrobial susceptibility/resistance pattern needs constant and strategic research to detect the emergence and spread of the antimicrobial resistance (Kumar *et al.,* 2011).

In addition to the increasing antibiotic resistance of *S. aureus*, there is danger of risk to human health due to consumption of milk having drug residues. Therefore, the findings of bacteriological analysis of mastitic milk samples, regarding the prevalence of udder pathogens and the level of antimicrobial resistance, of mastitis causing pathogens can be beneficial for the implementation of guidelines for prudent use of antibiotics (de Jong *et al.,* 2018). The antimicrobial resistance developed by the pathogens is one of main reasons of low cure rate of mastitis (Gao *et al.,* 2012). Hence, the findings of *in vitro* antimicrobial(s) susceptibility testing is an important diagnostic test for the selection and recommendation of most appropriate antimicrobial agent(s) for therapeutic intervention (Schwarz *et al.,* 2010). Considering above points, the present study was designed for the analysis of clinical mastitis cases and antibiotic sensitivity testing of staphylococci associated with mastitis.

Materials and Methods

Bacteriological examination of milk samples

A total of 565 quarter milk samples of 142 cattle received in College Central Laboratory were included in the present study. Animals having history of visible changes in udder secretions *viz.* flakes or clots, discolouration, watery consistency, abnormal taste, and/or swelling, oedema and pain in mammary glands and other clinical signs of anorexia, depression and fever i.e., suffering from clinical form of mastitis were included in the study.

Isolation and identification of bacteria

The milk samples were subjected for isolation of bacteria by inoculation of 10 µl thoroughly mixed milk of each quarter on 5% defibrinated sheep blood agar and MacConkey Lactose agar plates (Carter *et al.,* 1995). The inoculated plates were incubated at 37°C for 16-18 hrs. Growth of micro-organisms was identified on the basis of colony morphology, Gram's staining and catalase test.

Antimicrobial susceptibility testing of staphylococci

Antimicrobial susceptibility testing of 69 randomly selected staphylococci isolates was determined through disc-diffusion method on Mueller-Hinton agar by using eighteen commercially available antibiotic discs, as per method of Markey *et al.,* (2013).

Results and Discussion

Parity-wise occurrence of clinical mastitis

Highest number of clinical mastitis cases were recorded in 4th parity (34.51%), followed by 3rd (29.58%), 5th (14.08%), 2nd (7.75%), 1st (6.34), 7-12th (4.23%) and least number of cases were recorded in 6th parity (3.52%). These findings are in line with Sharma *et al.,* (2018a) and Seyoum *et al.,* (2017), who reported highest occurrence of mastitis in 3rd and 4th parity in dairy cows. High occurrence of mastitis in 3rd and 4th

parity might be due to high milk production in 4th parity along with the increase in diameter of teat canal and decreased tonicity of sphincter, therefore the environmental pathogens may have easy access to enter in teat canal and proliferate in mammary tissues (Constable *et al.,* 2017). Also, the breakdown of keratin layer of streak canal barrier with increasing age may be responsible for increased vulnerability to mastitis (Joshi and Gokhale 2006). Least number of clinical mastitis cases in 6th and 7th and above parity may be due to rearing of less number of animals in higher parity by the owners due to decrease in production potential or any incurable disease which lead to culling of animals before reaching to 6th and higher parity.

Occurrence of clinical mastitis with respect to post-partum of lactation

Highest number of clinical mastitis cases were recorded in 1st month of lactation (33.10%), followed by 2nd (16.20%), 3rd (19.01%), 4th (10.56%), 6th and above than 7th (7.75% each) and least number of cases were recorded in 5th month of lactation (5.63%). These findings are in corroboration with Sarba and Tola (2017) who reported 45.9% cases and early stage of lactation (less than 5 months) and 37.7% cases in late stage (more than 5 months) of lactation. Also, Tufani *et al.,* (2012) reported high incidence in early stage of lactation (52.38%), followed by mid (26.98%) and late stage of lactation (20.63%).

Highest number of cases in first month of lactation just after parturition may be due to increase in oxidative stress and low levels of antioxidant defence mechanism just after parturition (Sharma *et al.,* 2011). In contrary to this, Zeryehun and Abera (2017) reported maximum prevalence of mastitic in mid stage of lactation (61.3%), followed by early (50.7%) and late stages (48%) of lactation in animals of dairy farms. Also, Joshi and Gokhale (2006) reported maximum number of cases in 4th and 5th months of lactation, followed by 1st to 3rd months post-parturition. They explained that it may be due to physiological stress on animals attributed to high milk production.

Occurrence of clinical mastitis based on course of disease

Based on course of disease, the cases of clinical mastitis were categorized in peracute, acute, subacute and chronic. The cases of acute mastitis (42.96%) were found to be highest in occurrence, followed by subacute mastitis (40.14%) and chronic mastitis (16.90%) cases. Tufani *et al.,* (2012) also reported highest cases of acute cases (55.56%) followed by subacute (25.40%) and chronic cases (19.05%) of mastitis. Chronic mastitis may flare up occasionally or may persist in subclinical form (Constable *et al.,* 2017).

Frequency of isolation of bacterial pathogens

A total of 394 (69.73%) quarter milk samples were found culturally positive out of 565 milk samples included in the study. Distribution frequency of bacterial isolates is depicted in table 1. All the Staphylococci exhibited positive reaction for catalase test and observed as Gram-positive cocci arranged as bunches of grapes. *Staphylococci* spp. (60.04%) was found to be the main etiological agent associated with clinical mastitis in the present study (Table 1).

This is in line with the findings of Tufani *et al.,* (2012), Kaur *et al.,* (2015), Elemo *et al.,* (2017) and Sharma *et al.,* (2018b) from mastitis cases. *Staphylococci* spp. is pervasive in nature and may be present in bedding material, body surface of animals including teat and external orifice and farm equipments; including teat cups of milking machine and persist for a long period in the environment resulting in easy access to gain entry in teat canal. *Staphylococci* spp. are invasive micro-organism, establishes themselves in mammary glands through colonizing into deep udder parenchyma. Transmission mainly occurs between animals during milking process mainly through the milker's hands or milking machines (Sharma *et al.,* 2007 and Constable *et al.,* 2017).

Occurrence of *Streptococci* spp. (24.02%) was found to be second highest among the isolated bacteria in the present study. These findings are in accordance with Tufani *et al.*, (2012), Kaur *et al.*, (2015), Bhat *et al.*, (2017), Sharma *et al.*, (2018b) and Tomazi *et al.*, (2018). While contrary to our findings some researchers (Dubal *et al.*, 2010 and Kurjogi and Kaliwal 2011) reported *E. coli* as the second highest occurring organism in mastitis cases.

Occurrence of *E. coli* was 10.26% in the present study, which is in agreement with Dubal *et al.*, (2010) and Bhat *et al.*, (2017); whereas Kurjogi and Kaliwal 2011 reported 21.08%. However, Tomazi *et al.*, (2018) reported lower frequency of *E. coli* isolates (6.6%), in their study.

Occurrence of *Klebsiella* spp. was found to be 2.84% in the present study and this finding is in line with Kaur *et al.*, 2015 and Sharma *et al.*, 2018b. While non-existence of *Klebsiella* spp. was reported in findings of Bhat *et al.*, (2017) and Tomazi *et al.*, (2018).

Very low occurrence of *Diplococci* spp. (1.97%), *Corynebacterium* spp. (0.66%) and *Pseudomonas* spp. (0.22%) was found in the present study of bovine clinical mastitis cases. Kaur *et al.*, (2015) reported high occurrence (25%) of *Corynebacterium* spp. in their findings. While Bhat *et al.,* (2017), Elemo *et al.*, (2017) and Tomazi *et al.*, (2018) did not reported any *Corynebacterium* spp. in their findings. Kaur *et al.*, (2015) reported prevalence of *Bacillus* spp. (4%) and *Pseudomonas* spp. (6%) in their study. Sharma *et al.*, (2018b) also reported the presence of *Pseudomonas* spp. in the mastitic milk samples from bovines. While non-existence of *Bacillus* spp., *Diplococci* spp. and *Pseudomonas* spp. was reported by Bhat *et al.*, (2017), Elemo *et al.*, (2017) and Tomazi *et al.*, (2018).

The variations in occurrence of pathogens may change with time, diverse geographical regions, dissimilar type of treatment practices in the region and the management and hygienic practices. Therefore, bacteriological examination should be done to know the particular pathogen associated with mastitis case and to choose the most appropriate antimicrobial against the mastitis causing pathogen.

Antimicrobial resistance of Staphylococci spp. isolated from clinical mastitis

In the present study highest resistance was found in the antimicrobials of penicillin group; ranging from 85.51% in amoxicillin to 95.6% in cloxacillin, while low resistance in methicillin (11.59%) was observed (Table 2). These findings are in line with Kaur *et al.*, (2015), Sharma *et al.*, (2015), Ganai *et al.*, (2016) and Wang *et al.*, (2016).

While Feng *et al.*, (2016) reported low level of resistance against methicillin 2.27%, although they reported high resistance towards penicillin (84.09%). Contrary to findings of the present study Piotr *et al.*, 2013 reported low level of resistance in amoxicillin (17.9%), ampicillin (22.8%) amoxicillin (17.9%), ampicillin (22.8%) and penicillin (23.6%) against *Staphylococcus aureus*.

Table.1 Distribution frequency of bacterial isolates from clinical mastitis cases in cattle

Sr. No.	Bacteria isolated	Bacterial isolates (n=458)	Percentage
1	*Staphylococci* spp.	275	60.04
2	*Streptococci* spp.	110	24.02
3	*E. coli*	47	10.26
4	*Klebsiella* spp.	13	2.84
5	*Diplococci* spp.	9	1.97
6	*Corynebacterium* spp.	3	0.66
7	*Pseudomonas* spp.	1	0.22
	Mixed infections	64*	13.97*

Table.2 Antimicrobial susceptibility of *Staphylococci* spp. (n=69) isolated from clinical cases of cattle

Antibiotic class	Antimicrobial drug	Antimicrobial susceptibility					
		Resistant		Intermediate		Sensitive	
		n	%	n	%	n	%
Penicillins	Penicillin	60	86.96	0	0	9	13.04
	Amoxicillin	59	85.51	0	0	10	14.49
	Ampicillin	60	86.96	0	0	9	13.04
	Cloxacillin	66	95.65	0	0	3	4.35
	Oxacillin	64	92.75	0	0	5	7.25
	Methicillin	8	11.59	12	17.39	49	71.01
Cephalosporins	Cefuroxime	12	17.39	12	17.39	45	65.22
	Cefoperazone	30	43.48	21	30.43	18	26.09
	Ceftriaxone	18	26.09	30	43.48	21	30.43
	Cefotaxime	13	18.84	35	50.72	21	30.43
Aminoglycosides	Amikacin	36	52.17	20	28.99	13	18.84
	Gentamicin	21	30.43	12	17.39	36	52.17
Amphenicols	Chloramphenicol	22	31.88	35	50.72	12	17.39
Fluoroquinolones	Enrofloxacin	58	84.06	0	0	11	15.94
	Levofloxacin	40	57.97	13	18.84	16	23.19
	Moxifloxacin	58	84.06	9	13.04	2	2.90
Tetracyclines	Oxytetracycline	65	94.20	0	0	4	5.80
Lincosamides	Lincomycin	30	43.48	0	0	39	56.52

n= number of isolates

Following the penicillins; oxytetracycline was found to be second highest resistant (94.2%) antimicrobial in the present study. Similar to this, Kumar *et al.*, 2010, Kumar *et al.*, 2011 and Feng *et al.*, (2016) also reported oxytetracycline among the most resistant antimicrobial in their findings, however, they reported low level of resistance 36.7%, 33.6% and 15.8%, respectively, against oxytetracycline in their findings. Among aminoglycosides, amikacin (52.17%) was observed as more resistant than gentamicin

(30.43%). Kaur *et al.,* (2015) also reported gentamicin (91%) as more sensitive as compare to amikacin (51%). Feng *et al.,* (2016) and Mahato *et al.,* (2017) reported low level of resistance towards gentamicin; 9.09% and 9.7%, respectively.

Among fluoroquinolones highest resistance was found towards moxifloxacin (84.06%), followed by enrofloxacin (84.06%) and levofloxacin (57.97%). Contrary to this, Sharma *et al.,* (2015) reported low resistance towards enrofloxacin (33.33%) and levofloxacin (22.22%) and Kaur *et al.,* (2015) observed 12% resistance towards enrofloxacin.

Cephalosporins were found to be least resistant antibiotics among the tested antimicrobials in the present study. Among cephalosporins, highest resistance was observed in cefoperazone (43.48%) while cefotaxime (13%) was least resistant in the present study. Similar to this, low resistance in ceftriaxone was reported by Ganai *et al.,* (2016) and Sharma *et al.,* (2015) 29.41% and 33.33%, respectively. Contrary to this, Sharma *et al.,* (2018b) reported high sensitivity of *Staphylococci* spp. towards cefoperazone (93.40%) and ceftriaxone (89.56%)

The remarkable increase in antimicrobial resistance is due to extensive use of same class of antibiotic for treatment purpose and irrational use of antimicrobials without prior antibiogram profiling of the causative agent (Kumar *et al.,* 2010). The level of resistance for different antimicrobials differs between the regions and choice of antimicrobials used for the treatment of animals. Penicillins and oxytetracyclins have been frequently used for treatment of diseases in field settings. Therefore, the huge use of antimicrobials might be responsible for increased resistance towards these antimicrobials.

The most common mechanism of resistance in *Staphylococci* spp. is β-lactamase production, which leads to resistance towards β-lactamase sensitive antimicrobials i.e. penicillin G and amino-penicillins (Taponen *et al.,* 2017).

High occurrence of mastitis needs appropriate strategies for control of this disease in cattle to minimize the economic losses. For control of this disease, awareness of the farmers is needed for use of teat dips and hygienic practices at farm. Antimicrobial sensitivity testing should be done before using the antimicrobials to animals. Cautious use of antibiotics should be done to decrease the pace of development of resistance in microbes.

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