**Occurrence of Clinical Mastitis in Cattle with Emphasis on Antibiogram of Staphylococi**

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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.

**Keywords**

Clinical mastitis, Cattle, Staphylococci, Antibiogram, Antimicrobial, Resistance

**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 microorganisms 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 microorganism, 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         | %         |
| 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     |

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.
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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