Annals of Biology **38** (2) : 306-310, 2022

Impact Score : 0.32 (Scopus)

Bacterial Clinical Mastitis Study and Antibiotic Sensitivity Assay of Bovine

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(Received : February 16, 2022; Accepted : April 9, 2022)

ABSTRACT

Bovine mastitis is one of the major problems faced by livestock owner and its treatment and control still remain a challenge. The study was planned to isolate and identify bacteria responsible for the most of the infections. Out of a total of 78 milk samples processed, 22 (28.2%) were found infected, out of which *Staphylococcus* spp. remained most predominant (59.1%) followed by *E. coli* (36.4%) and *Pseudomonas* spp. (4.5%). Antibiotic sensitivity assay revealed that *Staphylococcus* spp. isolates were 100% sensitive to oxytetracycline, followed by equal sensitivity (92.3%) to chloramphenicol, bacitracin, enrofloxacin and gentamycin. Maximum resistance was found against colistin followed by cephaperazone and ceftizoxime. Gram negative bacteria *E. coli* and *Pseudomonas* isolates were also found carrying resistance against bacitracin (88.9%) followed by amoxyclav (77.8%) and erythromycin (77.8%) besides ceftozoxime (66.7%).

Key words : Mastitis, bovine, antibiotic, *Staphylococcus*, *E. coli*

INTRODUCTION

Mastitis is the inflammation in the tissue of mammary gland which is characterized by changes in glandular tissue and invasion by pathogenic microbes (Tezera and Aman Ali, 2021). Due to inflammation, various problems arise like swelling, warmth, pain and redness due to infection and might also cause fever, malaise and chills that can be considered as diagnosis of mastitis. It is most common and frequently disease occurring that causes huge economic losses in dairy industries (Ashraf and Imran, 2018). There are wide ranges of microorganisms that are responsible for infection : mycoplasma, yeast, fungi and bacteria, where bacteria are prominent pathogens for mastitis. Somatic cell count (SCC) is widely used to identify the quality of milk, and for larger herds, the bulk milk somatic cell count (BMSCC) is used to estimate the prevalence of herd mastitis (Ashraf and Imran, 2018; Khasa *et al.*, 2020). The pathogens are classified as contagious and environmental pathogens, where contagious pathogens are *Staphylococcus aureus, Streptococcus agalactiae* and *Mycoplasma bovis* while the major environmental pathogens include *E. coli* and *Streptococcus uberis*. Mastitis is classified into two categories (1) Clinical mastitis and (2) Sub-clinical mastitis (Tezera and Aman, 2021). Sub-clinical mastitis is more prevalent in India (varying from 10-50%) than clinical mastitis (1-10%). From a total of Rs. 71.655 billions of economic losses in India, Rs. 41.511 billions were due to sub-clinical mastitis and Rs. 30.144 billions were due to clinical mastitis. The cost effective treatment, culling of animals and decreased milk production due to infection showed the emergence to maintain this disease burden over cattle. Apart from financial losses, there is major impact on human health too, because the antibiotics given to animals for treatment released in milk and consumed by human or infants will lead to serious health hazards.

The objective of our study was to illustrate the incidence of clinical mastitis in cow and buffaloes of Sonipat region and antibiotic sensitivity profile of isolated organism to provide effective and timely treatment to animals affected with clinical mastitis and also provide therapeutic intervention.

MATERIALS AND METHODS

The study was carried out in Sonipat district of Haryana, India. A total of 78 bovine milk samples presented to disease diagnostic laboratory during 2018-19, were tested in the study, which had shown clinical symptoms of mastitis. Milk samples were collected in

sterile vials and were inoculated on their respective medium with a sterile platinum loop under sterile condition. The plates were then incubated at 37°C for 24 h. The obtained colonies were then characterized through morphological and biochemical examination. Nutrient agar and mannitol salt agar were used to grow *Staphylococcus* spp. Nutrient agar media for *Pseudomonas* spp. Mac Conkey agar for *Enterobacter* spp. Golden and white colonies were obtained on nutrient agar, and yellow colonies were obtained on mannitol salt agar. For morphological characterization gram staining was done and stained smear was observed under microscope. Biochemical examination was done by IMVic test, catalase and cytochrome C oxidase.

All the isolates were subjected to *in vitro* antimicrobial sensitivity testing by using antibiotic disc (HiMedia Laboratories, India) by disc diffusion method. Briefly, two pure colonies were transferred to nutrient broth and incubated at 37°C till desired growth of inoculum, which was then streked on Muellar Hinton Agar plates with the help of spreader followed by placement of antibiotic disc. Plates were incubated overnight aerobically at 37°C. Zone of inhibition was recorded. Different 16 antibiotics disc (HiMedia Laboratory, India) used were Amikacin Ak30, Amoxyclav AMC30, Ampicillin AS10/10, Bacitracin B10, Chloramphenicol C10, Colistin CL10, Erythromycin E15, Gentamicin Gen10, Azithromycin AZM15, Cefoperazone CFS75/30, Ceftriaxone CIS30/15, Ceftizoxime CZX30, Levofloxacin LE5, Enrofloxacin Ex10, Moxifloxacin Mo5 and Oxytetracyclin O30.

RESULTS AND DISCUSSION

Among 78 samples, only 22 yielded bacterial growth on culture (Table 1), out of which eight were from cows (of 55 sampled) and 14 from buffaloes (of 23 sampled). Quarter-wise, isolation was highest from left hind quarter (LH; 9/22) followed by right hind (RH; 6/22), left fore (LF; 5/22) and right fore (RF; 2/22; Fig. 1). *Staphylococcus* was the most prevalent bacteria responsible for causing bovine mastitis based on the morphological and biochemical properties of colonies formed in culture out of these 22 samples (Table 2). The formation of white and golden colonies on nutrient agar, and characteristic grape like

Table 1. Species-wise detail of samples and isolated organism

S. No.	Species	Total number of samples	No. of positive samples	No. of negative samples
1.	Buffaloes	55	14	41
2.	Cows	23	8	15
	Total	78	22	56

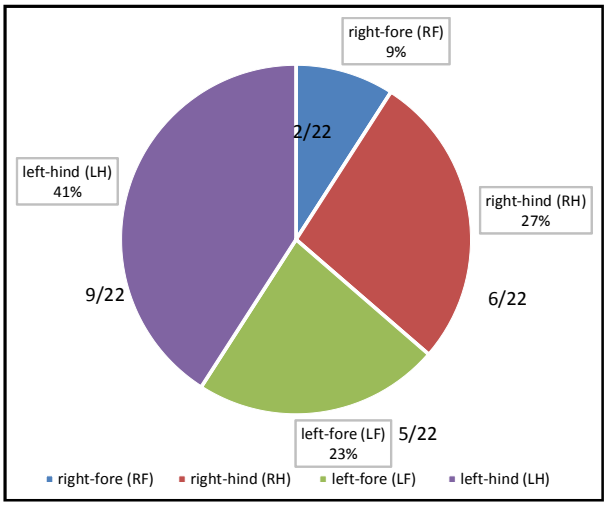

Fig. 1. Quarter-wise prevalence of bacterial isolates from clinical mastitis in bovine.

Table 2. Frequency of isolates from different bacterial species in mastitis milk samples

S. No.	Bacterial species	No. of isolates	Per cent
A. Gram positive bacteria			
1.	*Staphylococcus* spp.	13	59.1
B. Gram negative bacteria			
1.	*E. coli*	8	36.4
2.	*Pseudomonas*	1	4.5

pattern on gram staining indicated the growth of *Staphylococcus*. The pink coloured transparent smooth colonies on MC agar with pink coloured rod shaped gram stained smear confirmed the presence of *E. coli* isolates. The green colour pigmentation bacterial colonies on nutrient agar morphologically indicated the gram negative bacilli *Pseudomonas*. The confirmation was assured by biochemical characterization exhibiting the representative nature i.e. catalase and oxidase positive.

The total of 22 samples showing positive results for *Staphylococcus* spp., *E. coli* and *Pseudomonas* were further subjected to antibiotic susceptibility using 16 antimicrobial agents (Table 3). Among the antibiotics selected for the antibiogram profiling, oxytetracycline

Table 3. *In vitro* antimicrobial sensitivity pattern of bacterial isolates of mastitis bovine milk

Antimicrobial agents	Bacterial isolates								
	Staphylococci			*E. coli*			*Pseudomonas*		
	S	I	R	S	I	R	S	I	R
Amikacin	9	4	0	2	4	2	1	0	0
Amoxicillin	7	0	6	2	0	6	0	0	1
Ampicillin	12	1	0	3	2	3	1	0	0
Bacitracin	12	0	1	1	0	7	0	0	1
Chloramphenicol	12	0	1	6	1	1	1	0	0
Colistin	3	0	10	7	0	1	0	0	1
Erythromycin	8	2	3	1	1	6	1	0	0
Gentamycin	12	0	1	4	2	2	0	0	1
Azithromycin	6	1	6	3	3	2	1	0	0
Cefoperazone	3	1	9	2	1	5	1	0	0
Ceftriaxone	5	2	6	4	1	3	1	0	0
Ceftizoxime	5	0	8	2	0	6	1	0	0
Levofloxacin	10	1	2	4	1	3	1	0	0
Enrofloxacin	12	0	1	8	0	0	0	0	1
Moxifloxacin	6	3	4	4	0	4	1	0	0
Oxytetracycline	13	0	0	4	0	4	1	0	0

(100%), chloramphenicol (92.3%), gentamycin (92.3%), ampicillin (92.3%), bacitracin (92.3%) and enrofloxacin (92.3%) were most effective drugs against *Staphylococci* (Fig. 2). These expressed to be resistant to colistin (76.9%), cefoperozone (69.2%) and ceftizoxime (61.5%) followed by less resistance against amoxiclav (46.15%), azithromycin (46.15%) and ceftriaxone (46.10%).

The most effective drugs against *E. coli* were enrofloxacin (100%), colistin (87.5%) and chloramphenicol (75%), whereas *Pseudomonas* was sensitive against majority of antimicrobial agents. Both gram negative bacteria when recorded jointly, sensitivity in descending order were enrofloxacin (88.9%), colistin (88.9%) and chloramphenicol (77.8%), as resistance against enrofloxacin was observed in *Pseudomonas* strain. In combination, gram negative isolates were found carrying resistance against bacitracin (88.9%) followed by amoxyclav (77.8%), erythromycin (77.8%), ceftizoxime (66.7%) and cefoperazone (55.6%) and moxifloxacin (55.6%).

Haryana state is one of the leading milk producing states of the country and rearing of cattle and buffalo population helps in sustainability of farmers, growth of entrepreneurs and aids country in food security. One of the problems faced by this sector is losses due to mastitis in terms of milk loss and cost of treatment. In the present study, around 28.2% of the samples suspected for clinical mastitis were found to be infected with three bacterial species which were *Staphylococcus* spp. (59.1%) followed by *E. coli*

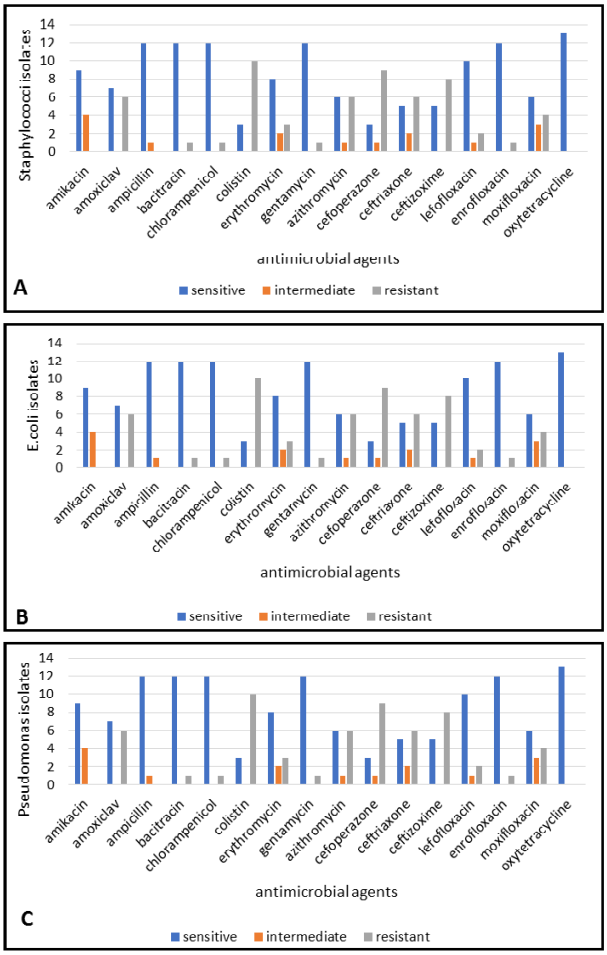

Fig. 2. Antimicrobial resistance among *Staphylococci* (A), *E. coli* (B) and *Pseudomonas* (C).

spp. (36.4%) and *Pseudomonas* spp. (4.5%). Mittal *et al.* (2018) reported highest prevalence of *Staphylococcus* spp. (53.9%) followed by *Streptococcus* spp. (29.7%), *E. coli* (10.2%) and *Pseudomonas* (0.5%) during period of 2015 to 2016, to which the present study was partially in agreement. In another study, Verma *et al.* (2018) also reported that *Staphylococcus* spp. was main mastitis causing pathogen. The study of Kurjogi and Kaliwal (2014), in Dharwad district of Karnataka observed that clinical mastitis prevalence rate ranged from 3.4 to 16.6%, slightly lower than the present study. The difference can be accounted for factor like selection of samples and geographical location. From other part of the world, in Brazil, prevalence was found highest for *Staphylococcus* spp. (32%) in sub-clinical mastitis, whereas *Pseudomonas* (9.5%) was more prevalent than *E. coli* (4.5%) in water buffaloes, which suggested that *Pseudomonas* spp. was capable of harming animal more sub-clinically rather than *E. coli*. which was responsible for more clinical mastitis. Chhabra *et al.* (2020) also reported that *Staphylococcus* was responsible for 63.54% of sub-clinical infection in Haryana which was in partial agreement of the present study, whereas the same organism caused most of clinical mastitis. Presence of *Staphylococcus* spp. from bovine mastitis milk in the present study along with other above reports needs to draw our attention since the bacteria can cause serious food poisoning in humans. Among gram negative bacteria, *E. coli* isolates showed that affected animal may be on risk of toxemia as observed in the study of Hagiwara *et al.* (2014). *Pseudomonas* spp. was found infecting only 27 out of 4378 tested mastitis milk samples of bovine, which suggested that it was less prevalent in this part of the country (Yadav *et al.*, 2020), which was in agreement with the present study, but still drug resistance of this bacteria had been widely reported which made it significant. In another study, Banerjee *et al.* (2017) reported 5.4% of sub-clinical mastitis was related to *Pseudomonas* spp. in West Bengal, India which was in agreement with the present study on clinical mastitis. Nimbalkar *et al.* (2020) reported 12.05% prevalence of clinical mastitis in Punjab, which was in partial agreement with the present study of Sonipat region of Haryana which was slightly higher considering farmers approaching the lab in later case. Quarter-wise prevalence in the present study showed that 40.9% of LH was most affected, whereas 9.1% LH was least.

Antibiotic remained one of the most preferred option in animal health sector for reduction of severity of disease and also control of the disease. Mastitis had been found linked with reproductive problem of animals (Kumar *et al.*, 2017), which made it more compulsive to treat such cases with antibiotics. The misuse of antibiotic and non-adherence to milk withdrawal period had been main problem identified in developing countries like India and Nigeria which needed to be dealt seriously to ensure food security and food safety and to achieve environmental and public health goals (Alhaji *et al.*, 2019; Mutua *et al.*, 2020).

Antibiotic resistance in the present study showed that most of routinely used antibiotics oxyteracycline, chloramphenicol, ampicillin, gentamycin and enrofloxacin were still effective against *Staphylococcus* isolated strain, whereas moderate level resistance was found against cephalosporin class of drugs (Cefoperazone, ceftizoxime and ceftriaxone). This suggested that usages of cephalosporin class of antibiotics were more compared to others, which was in agreement with the observation of Charaya *et al.* (2014), where sensitivity to chloramphenicol, gentamycin, amikacin and enrofloxacin was found higher. In another study, ceftriaxone (89.5%) and cefaperazone (93.5%) were found effective (Sharma *et al.*, 2018) in addition to oxytetracycline, ampicillin, amoxycillin and chloramphenicol.

Antibiotics found effective against gram negative *E. coli* and *Pseudomonas* in present study were enrofloxacin, chloramphenicol and colistin, which was in partial agreement with Charaya *et al.* (2014). Resistance was observed against bacitracin, amoxyclav, erythromycin and cephalosporin class of antibiotics in the present study which was on the disagreement with above study where cephalosporins were moderately sensitive. Observations of Mittal *et al.* (2018), were also similar to present study related to more sensitivity of chloramphenicol, gentamycin and enrofloxacin. Report of Yadav *et al.* (2020) that multiple drug resistance of *Pseudomonas* spp. isolated from mastitis cases was worrisome, where chloramphenicol and tetracycline were found ineffective.

CONCLUSION

It can be concluded that clinical mastitis of bovine is predominantly caused by *Staphylococcus* spp., *E. coli* and *Pseudomonas* in this region of Haryana. Most of the antibiotic works fairly against *Staphylococcus* but still cephalosporin class of antibiotics are getting in zone where they would not yield desired result. Similarly, gram negative bacteria also found carrying resistance against erythromycin and cephalosporines. Hence, these antibiotics need to be used only after antibiotic sensitivity testing.

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