Prevalence and Antimicrobial Susceptibility of Bacteria Isolated From Bovine Mastitis

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ABSTRACT

Mastitis is an inflammatory reaction of the udder tissue which is caused by the bacterial infection. Bovine mastitis is one of the devastating disease causing huge loss to the dairy industry worldwide. The present study investigated the current status of clinical and subclinical mastitis among dairy cattle in Dharwad district, Karnataka. The prevalence of mastitis was assessed by surf field mastitis test (SFMT), measure of pH and also based on the result of bacteriological evaluation of milk samples. A total of 185 isolates were recovered from 120 milk samples. The major pathogens isolated from the milk samples were S.aureus, E.coli, coagulase negative staphylococci (CNS), Streptococcus species, Bacillus subtillis, Serratia marcesens and other Bacillus species. Antibiogram studies were also conducted for the isolates by using fourteen antibiotics like kanamycin, cloxacillin, rifampicin, ampicillin, penicillin-G, carbencillin, chloramphenicol, cephalothin, tetracycline, trimethoprim, polymyxin-B, streptomycin, gentamicin and amikacin which were used frequently in this area for the treatment of mastitis and tetracycline was found to be more effective antibiotic among all the tested antibiotics.

Key words- Bovine mastitis, Prevalence, Antibiogram, Inflammation and Subclinical.
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INTRODUCTION

Mastitis is an inflammation of the mammary gland of the cattle's accompanied by physical, chemical and bacteriological changes in milk and glandular tissue. Bovine mastitis is one of the devastating diseases causing huge loss to the dairy industry worldwide. The costs associated with mastitis are innumerous and include antibiotic treatment, reduced milk quality, reduced milk yield, increased culling rate and the public health is affected due to infections caused by consumption of mastitis afflicted milk, bacteria have long been recognized as an important causal agent in bovine mastitis and also can transmit to humans through horizontal infection. Milk drawn from an infected cow can transmit pathogenic bacteria to humans through food chain

229

which is dangerous to the public health earlier it was discovered that milk can transmit tuberculosis, brucellosis, diphtheria, scarlet fever and Q fever to humans. However, these diseases can be controlled by a pasteurization technique, but a variety of bacteria still contribute to illness and diseases outbreak. The milk from an infected animal is the main source of pathogenic bacteria [3] and some bacterial toxins produced in the milk cannot be destroyed by heating or drying (National mastitis council, 1996). Therefore, the present investigation was undertaken to evaluate the microbiological and pharmacological aspect of the treatment of bovine mastitis

MATERIALS AND METHODS

Source of milk samples
The lactating cows of the dairy farms of the Dharwad district, Karanataka region have been examined from dairy herds in different small and large scale farms. A total of 150 animals were considered in the study which include holstein Friesian, Jersey and indigenous cows. Milk samples were collected aseptically for bacteriological studies as suggested by Honkanen-Buzalski [5]. Prior to sampling, the first streams of milk were discarded, and teat ends were disinfected with cotton swabs soaked in 70% alcohol and allowed to dry.

Time of milking
The time of milking was done at 4pm from the above described cows from various privately organized dairy herds. The milk samples were collected in a sterile polypropylene containers and brought to the laboratory of the Post Graduate Department of studies in Microbiology and Biotechnology, Karnatak University, Dharwad for further analysis.

Categorization of mastitis
mastitis was classified into two types that is sub clinical where no symptoms are visible and clinical mastitis – in which symptoms like swelling, redness, hardness and rise in temperature has been observed.

Analysis of milk samples
A total of 150 milk samples were screened for the detection of mastitis by surf field mastitis test [14]. The formation of gel was used for screening of mastitis. In this study pH was used as one of the important parameter as the indicator of mastitis where the normal milk pH is 6.6 – 6.7 where as milk with higher pH indicates the positive test for mastitis [12].

 In the present study bacteriological examination was carried out for the detection of mastitis in which from each sample, 0.01 ml of milk was cultured on nutrient agar and incubated for 48 h at 37°C. The plates were examined for growth, colony morphology and haemolytic characteristics on blood agar medium. Isolated organisms were streaked on differential medium and identification was carried out by conventional methods like grams staining, microscopic observation and biochemical tests.

Antibacterial susceptibility testing
Antibiotic susceptibility screening was done as per the guidelines of National Committee for Clinical Laboratory Standards (NCCLS). Kirby- Bauer's disc diffusion technique was adapted

for antibiogram. The antibiotic discs and Mueller- Hinton Agar were purchased from Hi-Media, Mumbai. The plates were prepared as per the manufacturer's instructions and checked for sterility by incubating the plates overnight at 37°C. The antibiotics discs were kept at room temperature for 1 hour before use.

RESULTS

Analysis of milk (Table 1)
The results of the present study showed prevalence of clinical and sub-clinical mastitis as 8 and 72% respectively and 20% as healthy animals. A total of 150 milk samples were screened for mastitis by surf field mastitis test (SFMT) and pH test out of which 120 milk samples showed positive for mastitis.

Bacteriological analysis (Table 2)
The bacteriological analysis of the present study showed that 185 bacteria were recovered from 120 milk samples. The predominant species were *Staphylococcus aureus* 28.10% followed by *E.coli* 21.08%, coagulase negative staphylococcus 18.91%, *Streptococcal species* 15.13%, *Bacillus species* 7.56%, *Serratia marcesens* 5.94% and *Bacillus subtillis* 3.24%.

Antibacterial susceptibility test (Table 3)
The antibiotic susceptibility test revealed that highest number of *staphylococcus aureus* were susceptible to tetracycline 76.92% and the least number of *S. aureus* were susceptible to pencellin G 1.92%.

The antibiotic susceptibility tests showed that highest number of E.coli were susceptible to chepalothin and tetracycline 76.92% and least susceptible to pencellin G 38.46%.

The antibiotic susceptibility test revealed that highest and the least number of coagulase negative staphylococcus were susceptible to kanamycin 80% and pencellin G 5.71% respectively.

The antibiotic susceptibility test suggested that highest number of *Streptococcas species* were susceptible to tetracycline and chloramphenicol 100% and least susceptible to polymyxin B 14.28%.

The antibiotic susceptibility test indicated that highest number of other *bacillus species* were susceptible to kanamycin 85.71% and least to carbencillin, polymyxin B and pencellin G 14.28%.

In antibiotic susceptibility test *Serratia marcesens* showed highest susceptiblity to ampicillin 81.81%, and least susceptible to carbencillin, polymyxin B and pencellin G 27.27%.

The antibiotic susceptibility test suggested that highest number of *Bacillus subtillis* were susceptible to kanamycin, tetracycline and ampicillin 83.33% and least to carbencillin 0%.

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DISCUSSION

In the present study 120 milk samples were shown positive for mastitis. The findings of the present study are in accordance with the work of Abid Hussin *et al.,* [6]. When milk from mastitis infected animal is mixed with anionic detergent solution such as CMT or SFMT reagent, a chemical reaction causes gel formation and the gel formation was divide into four types moderate (+), severe (++), more severe (+++) and very severe (++++) [14] and pH of normal milk is 6.6 or 6.7 when infection is present the pH increase to alkalinity which approximates to that of blood (7.2±0.2). Both SFMT and pH test are a good and economic methods used for the diagnosis of mastitis and farmers should use these techniques to test the animal before purchasing and avoid buying the animals which shows positive for these test. In the present study the high prevalence *staphylococcus aureus* followed by *E.coli*, *coagulase negative staphylococcus*, *streptococcal species*, *bacillus species*, *Serratia marcesens* and *bacillus subtillis* were relatively similar to the findings of sumathi *et al.,* [16], waage *et al.,* [18] and [8]. however results of the present study are comparable to the findings of the Joshi and gokhale, [7], Arshad *et al.,* [1] and Moroni *et al.*, [13].

Mastitis is a result of interaction between three elements like bacteria, cow and environment. In the present study the prevalence of staphylococcus species may be due to the incomplete milking and especially when it is associated with the painful lesions or any wounds on the outer surface of the udder. Staphylococcus is an opportunistic pathogenic bacteria which survive on the skin of the udder and can infect the udder via teat canal or any wound. Further, the prevalence of enterobacteria species in the present study may be due to the poor hygienic condition in the herds and this infection is becoming more and more frequent which tends to follow the infection of staphylococcus species. The prevalence of streptococcal species may be due to poor dairy practicing methods, it is the contagious organism which infects other healthy animals in the herds. The prevalence of bacillus species in the present study may be due to the environmental factors like soil water and manure, these are the main source of bacteria and when animals are exposed to water, soil and manure these bacteria infect animals via teat canals. Similar findings showed that higher incidence of *E.coli* may be due to poor hygienic conditions as *E.coli* originate from the cows environment and infect the udder via the teat canal [16]. Moreover, staphylococci, pseudomonas and mixed growth were the second after *E.coli*. their presence was also an indication of sub-standard hygiene of farm management [15].

Therefore, the above findings indicate that mastitis can be controlled by hygienic conditions in the herds like keeping the animals away from the stagnant water, cleaning manure, use of germicidal solution for washing udder before milking and culling of infected animals.

Further in the present investigation antibiogram studies were also conducted for the isolates by using fourteen antibiotics which were used frequently in this area for the treatment of mastitis and tetracycline was found to be more effective antibiotic among all the tested antibiotics against all the bacteria isolated in the present study followed by kanamycin, cloxacillin, chloramphenicol, ampicillin, streptomycin, gentamicin, rifampicin, cephalothin, amikacin, trimethoprim, penicillin-G, carbencillin and least was polymyxin-B.

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The susceptibility of bacterial isolates in the present study was comparable to the existing reports by Mekonnen *et al.,* [11]. Whereas Sudhakar *et al.,* [15] have reported that ciprofloxacin was the most effective antibiotic against bacteria isolated from cattle and buffaloes. Dhakal *et al.,* [2], Kumar and Sharma [9] have found that highest sensitivity of bacteria were shown to enrofloxacin and gentamycin, similar findings have been reported by Sumathi *et al.,* [16] in which gentamycin was found to be most effective antibiotic. In contrast to these findings, it has been reported that 64% of the isolates, from mastitis milk were sensitive to tetracycline and 52.8% to ampicillin [4]. Recently it has been reported that the highest number of *S.aureus* isolated from bovine mastitis were susceptible to ceftriaxone [17]. It has also been showed that highest numbers of isolates of coagulase negative staphylococci isolated from bovine mastitis were susceptible to ceftriaxone [8].

Table1. Prevalence of clinical and subclinical mastitis

Cases	Infection percentage	Number of isolates
Healthy	20	-
Clinical	8	56
Sub clinical	72	129

Table 2. Prevalence of different bacterial isolates from the bovine mastitis milk sample

Sl. No	Bacterial species	No of isolates Subclinical	clinical	Total Percentage
1	*Staphylococcus aureus*	42	10	28.10
2	*E.coli*	16	23	21.08
3	*Coagulase negative staphylococcus*	27	8	18.91
4	*Streptococcal species*	21	7	15.13
5	*Serratia marcesens*	9	2	5.94
6	*Bacillus subtillis*	06	0	3.24
7	*Other bacillus species*	8	6	7.56

Table 3. Antibiotic susceptibility profiles of bacteria isolated from bovine mastitis

Bacterial isolates	n	K	CX	R	A	P	CB	C	CH	T	TR	PB	S	G	AK
S.aureus	52	48.07	21.15	53.84	15.38	1.92	48.07	73.07	63.46	76.92	63.46	7.69	67.30	42.30	40.38
E.coli	39	74.35	71.79	48.71	51.28	38.46	46.15	66.66	76.92	76.92	71.79	46.15	74.35	69.23	66.66
Coagulase negative staphylococcus	35	80	34.28	25.71	51.42	5.71	17.14	65.71	34.28	74.28	25.71	14.28	51.42	48.57	17.14
Streptococcal species	28	89.28	42.85	39.28	71.42	25	25	100	39.28	100	35.71	14.28	42.85	57.14	17.85
Serratia marcesens	11	72.72	45.45	72.72	81.81	27.27	27.27	54.54	36.36	72.72	36.36	27.27	54.54	45.45	45.45
Bacillus subtillis	06	83.33	50	33.33	83.33	66.66	0	66.66	33.33	83.33	16.66	16.66	66.66	50	50
Other bacillus species	14	85.71	35.71	64.28	71.42	14.28	14.28	42.85	21.42	64.28	21.42	14.28	42.85	35.71	35.71
Mean (%)		76.20	75.30	48.26	60.86	25.61	25.41	67.07	43.57	78.35	38.73	20.08	57.13	49.77	39.02

n- Number of isolates K-kanamycin, CX-cloxacillin, R-rifampicin, A-ampicillin, P-penicillin-G, CB-carbencillin, C-chloramphenicol, CH-cephalothin, T-tetracycline, TR-trimethoprim, PB-polymyxin-B, S-streptomycin, G-gentamicin and AK-amikacin

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CONCLUSION

The development of antibiotic resistance among bacteria that affects animal health is of growing concern in veterinary medicine. Antibiotic resistant bacteria in animals have also become a potential health risk for humans, as they are able to cause direct or indirect transmission of the infection. Therefore, establishing an antibiogram of pathogens is very important from the clinical and economic points of view. Therefore, the findings of the present study showed that tetracycline is the most effective antibiotic which can be used for control of bovine mastitis of holstein friesian, jersey and indigenous cows in the area of research. The chemotherapy of bovine mastitis is only one important aspect in the control of mastitis. It is well planed mastitis control program which includes an accurate diagnosis segregation of infected cows; preventive bacteriological testing and correct milking practice may be made to reduce the development of resistance in the bacteria. Further, the infection should be treated as early as possible by massive dosage, therapy should not be prolonged beyond the necessary, mixed infection can be treated by broad spectrum antibiotics like tetracycline to cure mastitis.

Acknowledgment

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