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STUDIES ON PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIAL ISOLATES OF MASTITIS IN CERTAIN AREAS OF ANDHRA PRADESH

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Abstract: The present study was conducted to determine the antibiotic sensitivity of mastitic milk samples obtained from cattle and buffaloes. Mastitis, the clinical condition in dairy animals leads to productive and economic loss. To reduce the development of resistance, duration of therapy and cost of the same, selection of effective antibiotic is of paramount importance to the mastogen.126 and 198 milk samples from cattle and buffaloes were collected and subjected to CMT and white slide test. The samples were also analysed for etiological bacteria by conducting cultural and biochemical tests. The ABST was conducted using 14 antibiotics to detect the sensitivity of mastogens. The results revealed that 8.73% and 6.06% for CMT, 91.27% and 93.94% for white slide test positive in cattle and buffaloe milk samples respectively. The cultural tests revealed that majority of *Staphylococcus* species followed by *Streptococcus*, *E.coli* and *Pseudomonas* sp. The antibiogram results showed highest sensitivity to enrofloxacin, ceftriaxone, chloramphenicol among the tested antibiotics.
Keywords: Antibiogram, Mastitis, CMT, ABST, Milk sample.

1. INTRODUCTION

Mastitis is one of the major economically important diseases in dairy industry, incurring an annual loss of 526 million dollars in India (Varshney and Naresh, 2004). Majority of the Indian dairy industry is in the hands of small and medium farmers, who don't focus much on detection of mastitis in its early stages. Milk is a nutritionally rich medium that supports growth of many pathogenic organisms, which under favourable conditions can lead to spoilage and health problems at the consumer end (Oliver *et al.,* 2005). The most common routes of bacterial entry to the udder is *via* farm environment particularly water source, milking vessels and hands of milkers (Robinson, 2005). Most of these pathogens could cause

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serious health complications making milk a potential vehicle for food borne diseases (Oliver *et al.*, 2005).

Numerous pathogenic organisms were reported to cause inflammation of the parenchyma of the mammary gland characterized by a range of physical and chemical changes in the milk along with pathologic changes in the glandular tissue termed as mastitis (Constable et al., 2017). It is a multi-etiological complex disease associated with the most expensive production disease inflicting major economic losses to dairy industry worldwide especially in developed countries (Seegers et al., 2003). Mastitis can be classified into two categories based on the clinical condition; sub-clinical mastitis and clinical mastitis. Sub-clinical mastitis is characterized by no visible changes in the quarter(s) of the udder and milk, or the existence of inflammation without presence of any gross signs (Sharma et al., 2012). Subclinical mastitis in cows is more prevalent in India (varying from 10-50%) than clinical mastitis (1-10%). The Average loss of milk yield due to subclinical mastitis is up to 17.5% (Joshi et al., 2006). Subclinical mastitis in dairy cows is gaining importance due to higher prevalence of about 15-40 times than the clinical form.

The economic consequences of bovine mastitis are not restricted only to the farm but expand beyond the dairy farm (related to production losses, treatment, culling and changes in milk quality) thereby have a significant impact on the farm business management. In India, the first comprehensive report on economic losses caused by mastitis was published in 1962 indicating annual losses of Rs. 52.9 Crores (Dandha & Sethi, 1962). However in later years with the launch of Operation flood, tremendous thrust was given on cross breeding program which resulted in tremendous increase in high yielding bovine population, leading to many fold increase in economic loss. The fact is evidenced from a recent report where in annual economic losses sustained by dairy industry in India, on account of udder infections have been projected about Rs. 6053.21 crores. Out of this, loss of Rs. 4365.32 crores (70%-80% loss) was credited to sub clinical version of udder infections (Dua et al., 2001). The annual economic losses due to bovine mastitis are estimated to be Rs. 7165.51 crores in India, out of which 57.93% (Rs. 4151.16 crores losses) has been attributed to subclinical mastitis. However, control of bovine mastitis is being constrained because of multiple etiological agents. Hence, a rapid, sensitive and specific diagnostic method capable of simultaneously detecting multiple causative agents is essential for surveillance and monitoring of udder health. Antibiotic sensitivity test is a useful tool for choosing the right drug for treating mastitis.

In view of the economic and productive impact of mastitis on dairy industry and also to reduce the risk of drug resistance, the antibiotic selected should be based on the sensitivity pattern instead of preformed ideas. Hence, the present study was hypothesised to screen the cattle and buffaloe milk samples for the different antibiotics available and explore the most senstitive one that could be incorporated in the treatment regimen of the disease.

2. MATERIALS AND METHODS

The work was carried out in the Department of Veterinary Pharmacology & Toxicology and Department of Veterinary Public Health and Epidemiology, CVSc,Proddatur, SVVU, Tirupati.

Drugs and Chemicals Used

Tryptic soya broth, Mueller Hilton agar, Antibiotic discs, 70% alcohol, hydrogen peroxide, mannitol salt agar, Eosin methylene blue agar, Grams stain.

Collection of milk samples

The milk samples procured from Krishna and Kadapa districts of Andhra Pradesh were collected in sterile screw capped vials from cattle and buffaloes suffering from mastitis.The milk samples were initially tested for california mastitis test (CMT) and white slide test.

Cultural and biochemical tests

The milk samples collected were inoculated into tryptic soya broth (TSB) and incubated for 18-24h. Later on samples were stained with Grams stain for preliminary screening of Gram positive amd negative bacteria. The samples were streaked on mannitol salt agar (MSA) for the confirmation of Staphylococcus species and EMB agar for E.coli. Biochemical tests IMViC were used to detect the presence of Pseudomonas sp. Catalase test was conducted to differentiate Staphylococcus from Streptococcus species.

Antiobiotic sensitivity test (ABST)

Fourteen antibiotics were used to screen the sensitivity pattern of mastogens in the collected milk samples using disc diffusion technique (Bauer et al., 1966). 18h cultures were spread over MHA impregnated with antibiotic discs and incubated at 37^0C for 18- 24h.The results were interpreted based on diameter of zone of inhibition as per manufacturers chart. Statistical analysis was performed using unpaired t-test to determine the level of significance at 5%.

3. RESULTS AND DISCUSSION

The results of antibiogram of clinical isolates is represented in Table 1 as given below. In the present study the cattle and buffaloe milk samples were screened initially using CMT and

white slide test. 8.73% of cattle milk samples and 6.06% of buffaloe milk samples showed CMT positive. In the white slide test conducted 91.27% of cattle milk samples and 93.94% of buffaloe milk samples showed positive result which were depicted in figure 1. In the bacteriological tests conducted majority of samples with a range of 34 to 48% showing staphylococcal infection followed by streptococcus species in cattle and buffaloes. Skin of udder and milk of infected mammary gland are major reservoirs of Staphylococcus *spp.* (Ranjan *et al.,* 2011) and it can penetrate deep into the tissue producing foci. Streptococcus *spp.* is obligatory parasites of mammary gland epithelium and could be affectively eradicated by detection, segregation, antimicrobial therapy and following hygienic milking practices (Gyles *et al.,* 2008). Higher prevalence of *E. coli* might be due to poor hygienic conditions, as it originates from farm environment and infects udder via teat canal (Sumathi *et al.,* 2008). In buffaloes, Pseudomonas species and mixed bacterial infections were of higher percentage when compared to cattle. Pseudomonas *spp.* is natural inhabitant of soil, water and sewage. Mastitis caused by this organism might be due to use of intra-mammary equipment and also use of contaminated water for washing the udder. The general behavior of buffaloes to stay in swampy environment along with wallowing behaviour might be the reason behind increased chances of contamination of udder *via* teat canal (Didugu et al., 2015). The results revealed that enrofloxacin and ceftriaxone showed sensitivity when compared to other antibiotics tested. The findings of the present study were in line with the reports of the authors (Sumathi *et al.,* 2008; Ranjan *et al.,* 2010; Harini and Sumathi, 2011).

4. CONCLUSION

Hence we conclude from our present study that Staphylococcus *sp* is the major etiological agent of mastitis and that enrofloxacin, ceftriaxone are the most effective therapeutic agents in treating mastitis in buffaloes. To enhance the awareness among the farmers regarding the indiscriminate usage of antibiotics is the need of the hour. The significance of antibiotic sensitivity test in choosing the appropriate drug of choice for treating mastitis should reach the farmers; which can avoid emergence of multi drug resistant strains and thereby improve public health.

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Table 1: Mean +SEM of sensitivity of tested antibiotics at 5% level of significance

Antibiotic	Buffaloe	Cattle
Enrofloxacin	50.68±0.03	39.11±0.04
Ceftriaxone+tazobactum	53.07±0.03	38.72±0.04
chloramphenicol	54.92±0.03	45.53±0.04
Gentamicin	60.69±0.03	55.00±0.03
Ciprofloxacin	60.69±0.03	51.52±0.04
Amikacin	52.23±0.03	55.00±0.03
Levofloxacin	64.44+0.03	112.27±0.02
Amox+sulbactam	197.51±0.01	144.92±0.01
Amoxicillin	65.29±0.03	69.72±0.03
tetracycline	228.06±0.01	112.27±0.02
Ofloxacin	82.52±0.02	59.34±0.03
Ceftriaxone	131.70±0.01	144.92±0.01
Tylosin	393.00±0.01	251.00±0.01
Cefoperazone+sulbactum	279.31±0.01	251.00±0.01

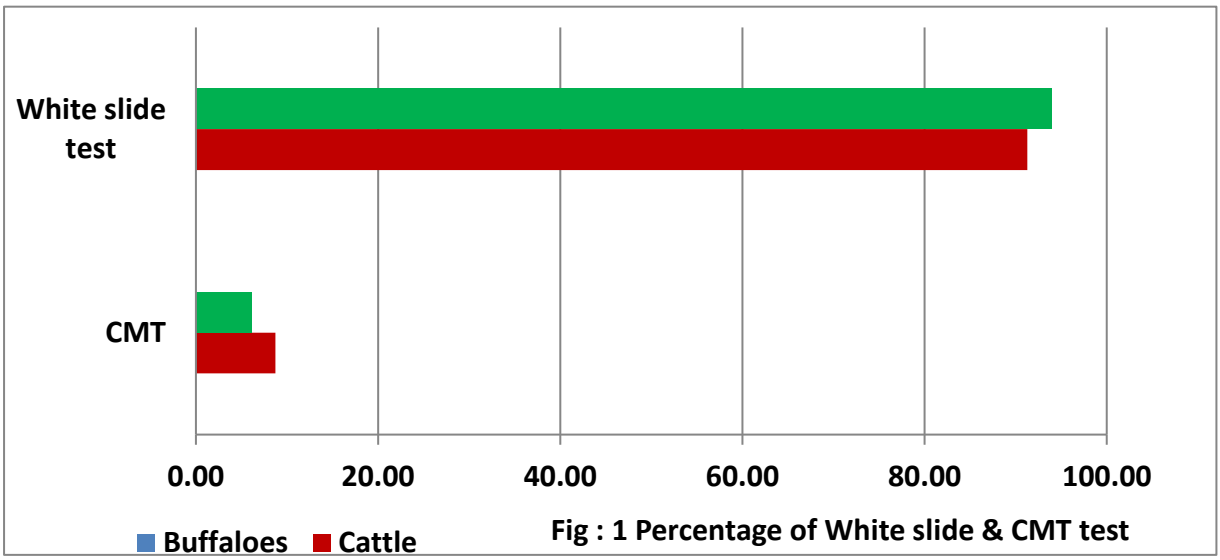

Figure 1: Percentage of White slide and CMT test

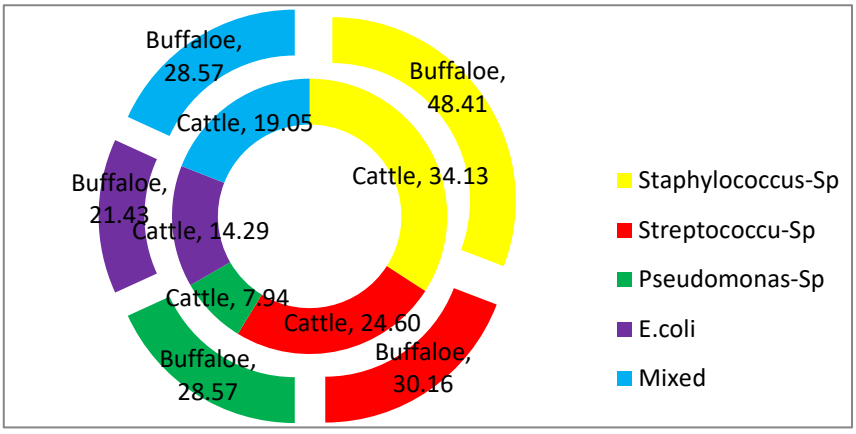

Figure 2: Percentage of causative bacteria in cattle and buffaloe milk samples

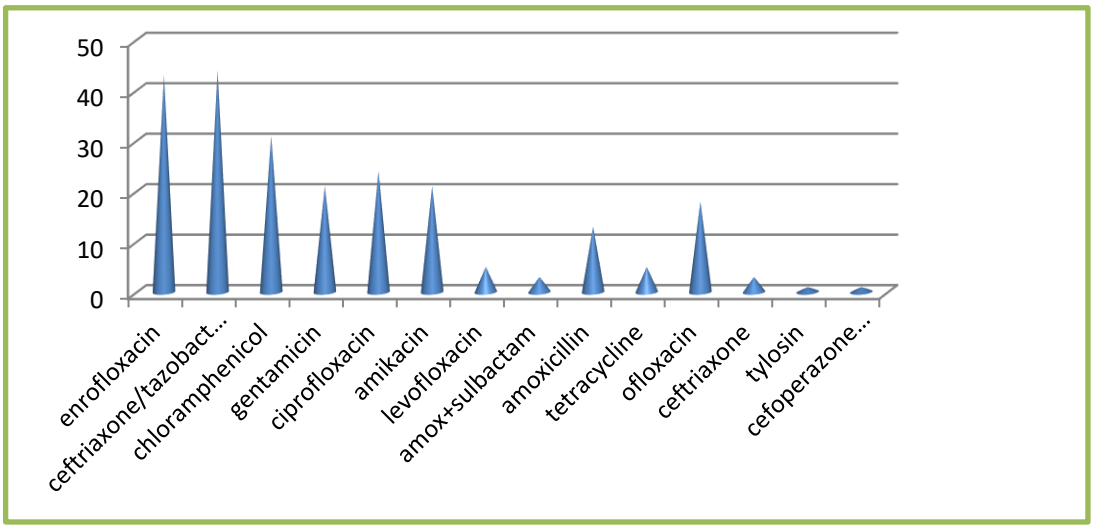

Figure 3: Antibiotic sensitive pattern in cattle milk samples