INCIDENCE OF MASTITIS IN COWS AND BUFFALOES UNDER DIFFERENT FARM CONDITIONS

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RANCHI- 834006 (JHARKHAND)

2009
THESIS

SUBMITTED TO THE
BIRSA AGRICULTURAL UNIVERSITY
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By

MAYUR KUMAR


IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

MASTER OF VETERINARY SCIENCE

IN

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Date:

(Dr. Mayur Kumar)

Place:

Certificate of the Major advisory and endorsement of the Head of the Department

Department of Veterinary Microbiology
Faculty of Veterinary Science & Animal Husbandry,
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CERTIFICATE

This is to certify that the thesis entitled “INCIDENCE OF MASTITIS IN COWS AND BUFFALOES UNDER DIFFERENT FARM CONDITIONS” submitted in partial fulfillment of the requirements for the Degree of Master of Veterinary Science (Veterinary Microbiology) of the Faculty of Post-Graduate Studies, Birsa Agricultural University, Ranchi (Jharkhand) is the record of bonafide research carried out by Dr. Mayur Kumar under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that such help or information received during the course of this investigation and preparation of the thesis have been duly acknowledged.

Endorsed:

(Dr.B.K.Tiwary)                              (Dr. Arun Prasad)
Chairman & Head of the Department           Major Advisor

Certificate of the Advisory Committee Members

CERTIFICATE

We, the undersigned, members of the advisory committee of Dr Mayur Kumar, a candidate for the Degree of Master of Veterinary Science with major in
Veterinary Microbiology have gone through the manuscript of the thesis and agree that the thesis entitled “Incidence of mastitis in cows and buffaloes under different farm conditions” may be submitted by Dr Mayur Kumar, in partial fulfillment of the requirements for the degree.

(Dr. Arun Prasad)
Chairman of the Advisory Committee

Members of the Advisory Committee

1. (Dr. B.K.Tiwary)

2. (Dr. B.K.Roy)

3. (Dr. K.K.Singh)

Certificate of approval by the Chairman of the Advisory Committee and External Examiner

CERTIFICATE

This is to certify that the thesis entitled “Incidence of mastitis in cow and buffaloes under different farm conditions” submitted by Dr. Mayur Kumar in partial
fulfillment of the requirements for the Degree of Master of Veterinary Science (Veterinary Microbiology) of the faculty of post-graduate studies, Birsa Agricultural University, Ranchi (Jharkhand) was examined and approved on (Date) ……………

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ABSTRACT

Mastitis is one of the oldest disease of milch animals. Today it stands second to FMD as a most challenging disease in high yielding dairy animals in India. Mastitis continues to be a major problem concerning dairy industry. It is recognized as one of the most costly disease affecting dairy cow. Therapeutic use of variety of drugs and antibiotics in mastitis appears to be ineffective (Pyorala et al., 1994). One of the striking consequences of indiscriminate use of antibiotic is development of drug resistance among mastitogen. Therefore an ideal therapeutic agent should have a minimum effect on milk quality and quantity and activate the defense system of mammary gland for optimum production of milk (Daley et al., 1992).

Hence it was thought necessary to conduct such investigation on incidence, prevalence and drug sensitivity pattern of prevailing organism to combat the present alarming problem.

The present study was undertaken to investigate the incidence of clinical and sub-clinical mastitis in cows and buffaloes under different farm conditions. For this study a total of 720 milk samples were collected of which 280 samples were collected from 70 buffaloes and 440 samples were collected from 110 cows. Out of 280 samples taken from buffaloes, 120 samples were from clinically affected and 160 samples were from apparently healthy animals. Similarly, out of 440 samples collected from cows, 160 samples were from clinically affected and 280 samples were from apparently healthy animals. All the samples were procured from organized farms, private farms and khatals of Ranchi.

All the samples were subjected to various physical examinations, cultural isolation & identification and drug sensitivity tests. The milk samples were also subjected to other tests such as California mastitis test, Somatic cell count, Methylene blue reduction test and Mastect kit test. A total of 282 bacterial isolates were found from 720 milk samples. The study revealed that the isolates of Staphylococcus species was highest followed by Streptococcus species, E.coli, Klebsiella species, Proteus species, Corynebacterium species and Yeast.
Incidence of sub-clinical mastitis was higher as compared to clinical mastitis in both cows and buffaloes under all farm conditions and the incidence were recorded higher in cows (42.50%) than in buffaloes (33.92%). The overall incidence of clinical and sub-clinical mastitis in cows were higher i.e. 31.25% and 48.92% as compared to those in buffaloes i.e. 21.67% and 43.13% respectively.

The prevalence of organism in cows and buffaloes was lower in organized farms (30%, 23.75%), slightly higher in Private farms (46.66%, 33.75%) and highest in khatals (59.16%, 40.83%), which might due to strict hygienic measures adopted in organized farms during and after milking.

Drug sensitivity revealed the overall effectiveness of Gatifloxacin and Levofloxacin against almost all isolates and Ceftriazone was found highly effective in case of grams negative organism.

So, Strict monitoring and surveillance measures in case of mastitis, drug sensitivity tests of organism causing mastitis and proper hygienic measures may be carried out in our state for the control of mastitis.
INTRODUCTION

Mastitis is one of the oldest disease of milch animals. The disease is complex in nature with multifaceted aetiopathogenesis. Mastitis is the term which denotes inflammation of the udder characterized by physical, chemical and microbiological changes in milk and pathological changes in glandular tissue of udder. The most important changes in milk include discoloration, presence of clots and presence of large number of leucocytes. In clinical cases there is swelling, heat, pain and indurations in the mammary gland.

Today it stands second to FMD as a most challenging disease in high yielding dairy animals in India. Mastitis continues to be a major problem concerning dairy industry. It is recognized as one of the most costly disease affecting dairy cow.

In India financial losses due to mastitis have been estimated to the tune of Rs 2809.32 crores per annum (Sirohi et al., 2001). Economic losses due to mastitis are incurred from:

1. Reduction in milk yield
2. Milk is discarded due to abnormal characters.
3. Culling of affected animals.
4. Cost of treatment
5. Cost of increased labour to care infected cow.
6. Decreased value of cow
7. Replacement cost

The incidence of sub-clinical and clinical mastitis in cow is reported to be higher 44.54% and 25.45% in comparison to that of buffaloes 39.12% and 21.95% respectively (Shike et al., 1998)

This disease is also important from public health point of view, presence of various bacteria in milk render it unfit for human consumption and leads to spread of
disease like tuberculosis, brucellosis, septic soar, throat, scarlet fever, and gastroenteritis.

Therapeutic use of variety of drugs and antibiotics in mastitis appears to be ineffective (Pyorala et al., 1994). One of the striking consequences of indiscriminate use of antibiotic is development of drug resistance among mastitogen.

Therefore an ideal therapeutic agent should have a minimum effect on milk quality and quantity and activate the defense system of mammary gland for optimum production of milk (Daley et al., 1992).

Hence this is thought necessary to conduct such investigation on incidence, prevalence and drug sensitivity pattern of prevailing organism to combat the present alarming problem.

Therefore the present investigation was undertaken with the following objectives.

1. To study the physical properties of milk samples from sub clinical and clinical cases of mastitis under different farm conditions.
2. Isolation of etiological agents from milk samples.
3. Identification of isolates on the basis of Cultural Characteristics
   - Morphology & staining
   - Biochemical profile
4. To carry out the drug sensitivity test of the representative isolates using various antibiotics.
Incidence:

Halverson et al. (1934) reported that in acute mastitis, the milk usually contains clots and its consistency is watery. In extreme cases the solid completely separate into a spongy mass which floats in an amber-coloured serum. But in chronic or sub-clinical, the udder appears superficially normal, flakes or clots may appear occasionally but the milk usually appears normal.

Parker (1942) reported that the prevalence of Staphylococci organism in bovine mastitis was becoming increasingly apparent. They found it in 76 percent of 2,296 bacteria positive milk samples examined for the presence of bacterial causing mastitis.

William Smith (1947) conducted studies on Coagulase-negative pathogenic staphylococci both physiologically and serologically to determine their relationship to Staphylococcus aureus. When 46 characters were studied and tallied, the 21 coagulase-negative pathogenic strains made up a heterogeneous intermediate group sharing not all the characters of S. aureus but appreciably more than S. epidermidis. Some characters among the coagulase-negative pathogens indicating a relationship to S. aureus were serotyping, lysostaphin sensitivity, growth rates, and endogenous respiration. Seventy-one percent of the coagulase-negative pathogenic strains were resistant to penicillin; of these, 80% were multiple antibiotics resistant.

Shah et al., (1958) examined 600 normal milk samples of which 50 Staphylococcus strains were identified as Staph. aureus and 94 as Staph. epidermidis.

Barber (1960) isolated Golden variants from two old laboratory strains of coagulase-positive Staphylococci which had become white and from three of six white variant cultures isolated, in the study of ‘aureus +albus ‘variation. These golden variants were isolated under conditions which are unfavourable to the growth of Staphylococci in the laboratory. They differed from the parent cultures in production of the factor which causes clumping in plasma, and showed a non-specific increase in resistance to various
antibacterial agents. Although it has long been accepted that pigment production by *Staphylococci* is not a reliable criterion of pathogenicity, 

Joshi and Dale (1963). Mannitol fermentation is a reasonably reliable method for the detection of coagulase positive *Staphylococci* in milk. This reliability can be improved if mannitol fermentation is carried out under anaerobic conditions.(2) Among hemolytic strains of *Staphylococci* isolated from milk, beta hemolytic *Staphylococci* predominate. Bovine and sheep blood agar plates give similar hemolytic patterns, but the hemolysis is more pronounced on sheep blood agar.(3) Gelatin liquefaction cannot be relied upon for the selection of coagulase positive *Staphylococci* in milk.

Zemelman and Longeri. (1964). Evaluated the pathogenicity of *Staphylococci* from bovine raw milk, the general characteristics of 775 strains isolated from 798 samples of milk were studied. Of 404 strains found to be coagulase-positive, 95.8% exhibited a deep-orange pigment, 76.5% produced ,8-hemolysin, 91.8% fermented mannitol, and 75% liquefied gelatin. Of 371 strains which gave a negative coagulase test, about 16% fermented mannitol and liquefied gelatin; none of these strains produced ,8-hemolysin. When results are grouped according to pigmentation and coagulase production, 3-hemolysin seems to be developed by pathogenic strains of *Staphylococcus aureus* only.

Raymond and Traub (1970) examined a total of 350 staphylococci isolated from various clinical sources for bound and free coagulase, fermentation of mannitol, and deoxyribonuclease. The economical coagulase-mannitol-agar method of Esber and Faulcomer was found to be suitable for the detection of free coagulase and mannitol fermentation. A significant number of coagulase- and mannitol-negative staphylococci proved to be deoxyribonuclease-positive.

Schalm *et al.* (1971) examined milk of cows and revealed the maximum number of cells (31 X 10^5 cells/ml) in the right Hind quarter followed by the left Hind quarter (2.8 X 10^5 cells/ml). The SCC of both the left fore and the Right Fore quarters were under normal range (0.60 X 10^5 cells/ml and 0.85 X 10^5 cells/ml respectively.

Morton and Cohn (1972) Tested a total of 504 clinical isolates of the family *Micrococcaceae* for coagulase, deoxyribonuclease, clumping factor,
and phosphatase to determine whether there is a correlation between the results of these tests and the pathogenicity of staphylococci. In the tests for coagulase production, it was found that either human or rabbit plasma could be used with broth cultures, whereas rabbit but not human plasma was satisfactory when microorganisms removed from solid culture medium were used.

Sudeshchander and Baxi (1975) tested all together 304 quarters of 789 cows with different indirect tests and their findings were compared with bacteriological examination and the various organisms isolated were Staphylococci (coagulase positive 49.52% and coagulase negative 19.05%), Streptococci (Haemolytic 10.48%) and non-haemolytic (5.71%), (0.95%) Corynebacterium, (2.86%) Pseudomonas, (2.86%) E. coli and (7.62%) other gram negative and positive rods.

Atherton and Newlander. (1977) The methylene blue reduction test is based on the fact that the color imparted to milk by the addition of a dye such as methylene blue will disappear more or less quickly. The removal of the oxygen from milk and the formation of reducing substances during bacterial metabolism causes the color to disappear. The agencies responsible for the oxygen consumption are the bacteria.

Sharma and Boro (1980) examined 30 milk samples from clinically affected quarters of cow in and around Khanapara (Assam) and revealed different organisms such as Staph. aureus (12), Streptococcus spp.(4), Corynebacterium pyogenes (5), Pseudomonas aeruginosa (3), E coli (6). Samples that contained mixed organisms of two types each were 6 in number.

Todorov et al. (1981) reported the prevalence of mastitis in 53.4 percent (average 13.5%) of 13880 cows in 40 farms. After testing it was found that 2382 quarters (4.28%) were infected, 3.23 percent had clinical mastitis, 58.14 percent subclinical mastitis, 12.55 percent exhibited the carrier state, 22.58 percent had secretary disturbances and 3.4 percent did not react to the test.

Chaudhry et al; (1982) reported that examination of 100 milk samples from 80 buffaloes with mastitis yielded 42 strains of Staphylococcus aureus, 6 of Staph. epidermidis, 22 of Streptococcus agalactiae, 6 of
Streptococcus dysgalactiae, 19 of *E. coli*, 11 of *Corynebacterium pyogenes* and 3 of *Pseudomonas aeruginosa*.

Funk *et al.*, (1982) reported environmental and physiological factors affecting mastitis at drying-off and post calving. They observed that 19.7 percent of all quarters tested (3987 cows) were infected at drying off. Mostly infections were caused by *Streptococcus agalactiae* (8.4%) and *Staphylococcus* spp. (7.8%). It was also observed that high yielding cows had fewer mastitic infections at drying off.

Guzman *et al.*, (1982) observed the prevalence of subclinical mastitis among Holstein Friesian cows to be 26.0 percent of quarters and 22.0 percent of cows each month. The prevalence over the year was 51.0 percent of quarters and 75.0 percent of cow.

Rahman *et al.*, (1982) reported that when antimicrobial susceptibility test was done on 252 strains of pathogens isolated from subclinical cases of mastitis in cows and buffaloes to nine chemotherapeutic agents by the disc diffusion method of testing, Neomycin, Chloramphenicol and Nitrofurantoin were found to be most effective against mastitis pathogens, while Penicillin and Streptomycin were found least effective.

Singh and Baxi (1983) examined 50 cows, 88 buffaloes and 20 goats. They found subclinical mastitis in 27 cows, 21 buffaloes and 14 goats.

Coni *et al.*, (1983) reported that Staphylococci were present in 58.0 to 64.0 percent bacteriologically positive milk samples (58.0 to 59.0 % of positive quarter samples) and streptococci was in 26.0 to 33.0 percent of milk samples (26.0 to 30.0% of positive quarter samples).

Kalorey *et al.* (1983) examined 128 cow and 25 buffalo milk samples and found that 45.6 percent were *Staph. aureus*, 31.7 percent were *Staph. epidermidis*, 9.24 percent *Corynebacterium* spp. The other pathogens isolated were Bacillus spp. (8), *Strept. Spp.* (7), *Pseudomonas* spp. (2), *E. coli*(2) and *Proteus* spp. (2).

Khalaf (1983) conducted a survey on quarter milk samples from 151 buffaloes to determine the prevalence of different types of mastitis. He observed that over prevalence rates of clinical and subclinical mastitis were 26.11 percent (12.08% quarter basis and 31.84 percent 11.65% on quarter basis), respectively. *Streptococcus agalactiae* (Lancefield group B). *Strept.*
dysgalactiae and Corynebacterium pyogenes. It was found that incidence of mastitis increased with increase in lactation number and age, and was also affected by stage of lactation.

Flinois and David (1984) examined cases of mild subacute mastitis and observed that 66 of them yielded 74 isolates, 47 percent of these were Streptococci, 23.0 percent Staphylococci, and 17.5 percent Escherichia.

Hussain and Mazni (1984) reported the incidence of mastitis in imported Jersey cows from Malaysia. They examined 107 cows (428 quarters) and mastitis was found in 95.0 percent of animals and 55.7 percent of quarters.

Ramachandra et al., (1984) examined bacteriologically 1262 milk samples from cases of mastitis and found Streptococci in 162 samples, Staphylococci in 136, Bacillus spp. in 56, Corynebacterium spp. in 86, Pseudomonas spp. in 86, E. coli in 56, Enterobacter spp. in 58, Proteus spp. in 4, and mixed infection in 36 samples.

Strehle (1984) reported that occurrence of subclinical bovine mastitis in Stuttgart region after screening of 300000 cows and found that 63.0 percent of 300000 cows had healthy udders, 21.0 percent had subclinical mastitis, 4.0 percent harboured bacteria without evidence of mastitis and 12.0 percent were giving abnormal milk (clinical mastitis). 45.3 percent of udder infections were caused by Staphylococci and 44.3 percent by Streptococci both types of bacteria were present in 4.0 and other bacteria in the remainder. The commonest Streptococci were Streptococcus uberis (28.0%) and Streptococcus dysgalactiae (27.0%), while Streptococcus agalactiae accounted for only 12.00 percent of Streptococcal infections.

Martel et al., (1985) isolated 515 Streptococci strains from cattle. Ninety five percent were from mastitic milk. The commonest mastitis strain was Streptococcus uberis (35%) followed by Streptococcus agalactiae (22%) and Streptococcus bovis (5.0%).

Njau and Kundy (1985) conducted a survey on etiological causes of mastitis and observed that over 80.0 percent of the infections were caused by Staph. aureus followed by Streptococcus agalactiae, Streptococcus dysgalactiae and Streptococcus uberis.
Sudhaona et al., (1985) examined 474 milk samples from cows with clinical and subclinical mastitis, half were culturally positive, 32.6 percent of these yielded *Staphylococci* in pure culture, 27.0 percent *Colliforms*, 15.0 percent *Streptococci*, 7.0 percent *Corynebacterium*, 4.6 percent yeasts, 2.5 percent Gram positive cocci and 1.2 percent *Pseudomonas* and remaining 10.8 percent shows mixed infections usually *Staphylococci* with *Colliforms*.

Bhindwal et al., (1987) reported that out of 81 isolates obtained the 2 commonest species were *Staphylococcus epidermidis* (38) and *Staphylococcus aureus* (32).

Pal et al., (1988) examined 81 cow and 56 buffalo milk samples and reported that 24 of 45 infected quarters (54%) contained *Staphylococcus* spp., 10 (22%) *Streptococcus* spp., 3 (20%) *E. coli* and 2 (4%) *Pseudomonas* spp.

Prabhakar et al., (1988) reported that bacterial isolates from 184 quarter of 94 cows and buffaloes with acute mastitis were found positive for *Staphylococcus aureus*, the most commonly isolated organism, *E. coli* the second most common causes of mastitis, followed by *Streptococcus* and *Pseudomonas* spp.

Chanda et al., (1989) reported microorganisms isolated from 113 milk samples 30.38 % (out of 372 samples) 65 isolates (57.52%) were *Staphylococci*, 40 isolates (35.40%) were *Streptococcus*, 6 isolates (5.31%) were *Corynebacterium* and 2 isolates (1.77%) were *E. coli*.

Parai et al., (1989) reported that when a total of 31 crossbred cows of an organized dairy farm, suffering from clinical mastitis in their 48 quarters were studied, the incidence of *Streptococcal* mastitis (41.9%) was highest followed by *Staphylococcal* (25.8%), *Staphylococcal* mixed (16.1%), *E. coli* (9.6%) and *Coccobacillus* (6.6%) mastitis in decreasing order.

Prabhakar et al., (1989) reported that bacteriological examination of milk from clinically affected quarters revealed that *Staphylococci* (64.8%) and *Escherichia coli* (22.2%) were the main causative organisms.

Rao et al., (1989) studied the incidence of clinical mastitis in bovines. A total of 10,079 bovines were screened. Among them 511 clinical cases of mastitis could be detected with an average incidence of 5.1 percent. The incidence was higher (48 percent) during winter months. Cultural
examination of the milk samples from 28 clinical cases revealed the presence of streptococci 35.7 percent (hemolytic 26.8 percent and non-hemolytic 8.9 percent); *Staphylococci* 30.3 percent (Coagulase positive 17.8 percent and coagulase negative 12.5 percent); *E. coli* 16 percent; *Pseudomonas* 10.8 percent and *Corynebacterium* 7.5 percent.

Rao et al.,(1989) studied the incidence of clinical mastitis in bovines. A total of 10,079 bovines were screened. Among them 511 clinical cases of mastitis could be detected. In vitro drug sensitivity of whole milk cultures indicated the highest sensitivity of Gentamycin, Nitrofurantoin and chloramphenicol; moderate sensitivity was observed to cephaloridine, ampicillin and streptomycin and the least sensitivity was observed to penicillin and cloxacillin.

Prabhakar et al. (1990) reported that only *Staphylococcus aureus* and coagulase negative *Staphylococci* were isolated from mastitis milk.

Rahman and Boro (1990) worked upon 115 milk samples from cows suffering from mastitis, 94 samples were culturally positive. Among isolates *Staphylococci* were found to be the main etiological agent. Other bacteria of significant importance were *Streptococci, Escherichia coli, Klebsiella* spp. and *Corynebacterium* spp.

Ramachandraiah et al., (1990) examined individual quarter milk of 80 Jersey cows for mastitis, 51 were found positive on CMT and cultural examination and *Staphylococcus* spp. was recorded as highest (52.9%) followed by *Streptococcus* spp.(20.3%) and *Pseudomonas* spp. (2.2%). Mastitis due to single isolates occurred in 90% while mixed infections recorded in 10% of the cases tested. Out of these mixed infections, combination of *Staphylococcus* spp. and *Diplococcus* spp. occurred in 45% of the cases.

Gupta et al., (1992) cultured milk samples from 1223 cows with bovine mastitis, the bacteriological examination revealed, 40.65 percent of Staphylococcus spp., followed by 29.35 percent of *Streptococcus* spp., 4.35 percent of *Corynebacterium pyogenes*, 3.35 percent of *Proteus vulgaris* and 2.28 percent of *Pseudomonas* spp.

Char et al., (1993) reported bacterial growth in (86.74%) clinical samples. Of these positive samples 75.28% had single infections and the
remaining 24.7% had mixed infections of gram positive and gram negative 
bacteria. *Staphylococcus aureus* predominated, followed by *E. coli* and *Streptococcus* spp.

Deluyker *et al.*, (1993) studied changes in milk yield associated 
with SCC and occurrence of clinical mastitis and differences in SCC with 
parity. During the 10-day period following a mastitis treatment, SCC 
differences between treated and control cows remained significant but 
became smaller with time and returned to the premastitis differences.

Kothe *et al.*, (1993) cultured 210 milk samples from 55 buffaloes 
with suspected mastitis and found 77 isolates of which 34 (44.2%) were 
*Staphylococcus* spp. (23 *S. aureus* and 7 *S. epidermidis*), 19 were 
*Streptococcus* spp. (S. uberis 6 and S. agalactiae 5), 10 were 
Enterobacteriaceae (*E. coli* 5), and 6 were mycotic agents.

Saini *et al.*, (1994) reported that the predominant species 
amongst 70 isolates were *Staphylococcus aureus* (34), coagulase negative 
*Staphylococci* (12), *Streptococcus* spp. (8) and *E. coli* (8).

Singh *et al.*, (1994) studied the incidence of subclinical mastitis 
in 8 farms of India (Punjab). It was 49.20 and 23.09% on an individual animal 
and quarter basis respectively. *Staphylococci* were the chief causative agents 
(73.08%) followed by *Streptococci* (14.61%) and *E. coli* (5.38%), 
*Corynebacterium*, *Proteus* and *Klebsiella* spp. were also isolated.

Prabhakar *et al.*, (1995) studied the incidence of clinical mastitis 
and management practices on 5 farms. 40 animals were affected with clinical 
mastitis having 76 affected quarters. *Staphylococcus* spp. (47.37%) were 
the major causative organism (34.21%) *S. aureus* and 13.16% coagulase 
negative *Staphylococci* followed by *Streptococcus agalactiae* (14.4%), *E. coli* 
(10.53%), *Pseudomonas* spp. (7.89%), *S. Pyogenes* (3.95%), *Klebsiella* spp. 
(3.95%), *S. dysgalactiae* (2.68%), *Proteus* spp. (2.63%) and S. uberis, no 
organism could be isolated from 2.69 percent of the quarters.

Wadhwa *et al.*, (1996) reported cases of clinical mastitis in 
Himachal Pradesh, milk samples were taken from 93 affected quarters (57 
cows) for bacteriological examination. The organisms isolated were species of 
*Staphylococcus* (68.83%), *Streptococcus* (16.88%), *E. coli* (7.8%), *Klebsiella* 
(2.59%) and *Diplococcus*, *Proteus* and *Corynebacterium* (1.3% each).
Chakrabarti et al., (1997) performed a trial involving 10 quarters of 6 dairy cows clinically affected with mastitis caused by *Staphylococcus* spp.

Muhammad et al., (1997) examined a total of 37 clinically affected mastitis quarters for bacterial isolates, *Staphylococcus aureus* was the most frequently (53.9%) isolated pathogen, followed by *S. Pyogenes* (15.4%), *S. agalactiae* (10.3%) and *S. hygiene* (7.7%).

Hwang et al., (1997) examined a total of 165 *Staphylococcus* species. Coagulase tube and slide tests were performed by rabbit plasma and human citrated plasma, respectively. The specificity and positive predictive value of slide coagulase tests, respectively, were as follows: rabbit, plasma slide test, 98.1 and 99.1%; Staphaurex Plus, 96.2 and 98.2%, and human plasma slide test, 54.7 and 82.4%. Three were no false positive or false negative results with five coagulase tests for 90 isolates of MRSA.

Shriram et al., (1997) examined subclinical milk samples from 106 buffaloes in the region of Nagpur, India. 24.05 percent milk samples were culturally positive, out of which 98 showed single and 3 mixed infections. Among the 104 isolates, coagulase positive *Staphylococcus* were the most prevalent, 58.65% were *Staphylococcus aureus*.

Umakanthan (1997) examined 14 cows severely affected with acute mastitis and found *Staphylococcus aureus* as causative organisms.

Madhu Babu et al., (1998) examined a total of 100 milk samples from cows and buffaloes affected with clinical mastitis, brought to animal Health Centre, identification of bacterial isolates were done and 55 percent of dominating etiological agent of *Staphylococcus aureus*, 16 percent of *Streptococcus* spp., 2.5% of *Corynebacterium* spp., 1.5% of *Pseudomonas* spp. and 1 percent of Bacillus spp was found.

Shike et al., (1998) examined 15 cows with clinical signs of mastitis and 54 cows with subclinical mastitis. Bacteriological examination revealed pure culture in 7 (31.82%) and mixed cultures in 15 (68.18%) of the subclinical infection and 9 (42.86%) and 12 (57.14%) respectively, from the clinical cases. *Staphylococcus* spp. was the most commonly found pathogen.

Shukla et al., (1998) recovered 247 isolates from 157 animals and identified Staphylococcus aureus as the most prevalent (60.32%), followed by *Streptococcus* spp. (31.98%), *E. coli* (1.22%), *Pseudomonas*
(2.42%), Pasteurella, Proteus mirabilis (0.40%), and Corynebacterium pyogenes (2.42%).

Swamy and Krishnamurthy (1998) reported that 148 isolates of Staphylococcus were obtained from 225 quarter samples, out of those isolates, 54.05 percent were S. epidermidis and 44.59 percent S. aureus. While the 2, 2 and 1 positive samples from the other sources respectively were S. aureus. The second highest prevalent organism was Escherichia spp. (28.39%) followed by Streptococcus.

Umakanthan et al., (1998) examined 20 randomly collected mastitic milk samples, cultured in the laboratory and found that 11 (55%) were infected with Staphylococcus spp., 3 (15%) with non-haemolytic Streptococcus, 3 (15%) with gram positive rods and a total of 15% with Colliforms, E. coli and Bacillus spp.

Dhote et al. (1999) examined milk samples from 1100 cows, 98.40% of the quarters were positive for single bacterial isolates of which most were Staphylococcus spp., Streptococcus spp. and Gram negative bacteria were also found. The results of sensitive tests of the isolates to 12 antibiotics showed that the majority of the Staphylococci and Streptococci were sensitive to Ciprofloxacin and were least sensitive to Amoxicillin and Penicillin.

Watts and Rossbach., (2000) conducted a study to find out the susceptibilities of Corynebacterium bovis and Corynebacterium amylocolatum Isolates from Bovine Mammary Glands to 15 Antimicrobial Agents. The MICs obtained with 46 strains of C. bovis against various antimicrobial agents were compared with 43 strains of C. jeikeium, a lipophilic corynebacterium isolated from humans. Phylogenetic studies have also determined that C. jeikeium is the organism most closely related to C. bovis.

Jae-Choon et al., (2000). The most common isolates from bovine mastitis were Staphylococcus sp., Streptococcus sp., and Corynebacterium sp and Escherichia coli, Examination of 16 antibiotics against these pathogens revealed that the incidence of antibiotic-resistant microorganisms were very high and that many of these isolates had multiple resistance to various commercially available antibiotics such as penicillin, ampicillin, erythromycin, streptomycin, norfloxacin, and tetracycline.
Soomro (2000): Out of 785 samples 109, 155 and 124 showed positive reactions on WST, BTBT and CLT respectively. The positive samples were graded as +(4), + + (8), + + + (12) and + + + + (16) according to the severity of reaction and their relative somatic cells were counted. The results revealed direct relation between SCC and intensity of chemical reaction. Ninety one percent of samples with positive reactions on all three tests had SCC over 200x103 which indicates that a SCC below 200x103 may be regarded as normal in buffalo.

Barbuddhi et al., (2001) reported that a total of 141 milk samples were collected aseptically for bacteriological studies, of these 48 milk samples were from clinical cases of mastitis. Out of 48 milk samples from clinical mastitis, 45 (93.75%) samples yielded bacterial growth. Subclinical mastitis was detected by CMT in 44.0 percent of milk samples which were bacteriologically positive also. The bacterial isolates revealed *Staphylococcus aureus* (23.25%) and *Streptococcus* spp. (11.65%). Other bacterial isolates were *Bacillus* spp. (8.14%), and *Proteus* (9.30%). Of 48 samples of clinical mastitis 27 (56.25%) yielded single causative agents and 21 (43.75%) had multiple etiological agents.

Banerjee et al., (2002) examined 512 milk samples and only 144 were found culturally positive. 11 samples yielded dual isolates, out of 155 isolated bacteria, 55 (54.84%) were *Staphylococcus* spp., 27 (17.42%) were E. coli and only 2 (1.28%) were *Pseudomonas* spp. Among 85 *Staphylococcus* spp.33 (38.82%) were found to be coagulase positive and 52 (61.18%) isolates were coagulase negative.

Bhattacharya (2002) reported that out of 72 culturally positive milk samples 64 (88.88%) cultures were single isolates whereas in 8 (11.11%) milk samples culture revealed mixed infection. In this study higher incidence of *Staphylococcal* mastitis (44.44%) was observed. Prevalence of other organisms such as *Bacillus* spp. 11 (15.27%), *Streptococcus* spp. 9 (12.5%) and *Condida* spp. 2 (2.77%) was also found.

Goswami et al., (2002) reported that a total of 48 bacterial isolates were obtained from milk samples out of which, *Staphylococcus* spp. (61) was predominant followed by *Enterobacteriaceae* (11), *Corynebacterium* (9) and *Streptococcus* spp. (6).
Kumar et al., (2002) examined 142 cows and 415 buffaloes, 59.86 and 66.27 percent respectively, were found positive in cultures and *Staphylococcus* spp. was the most prevalent organism.

Mohini *et al.*, (2002) subjected 81 milk samples to different indirect tests for the diagnosis of mastitis and cultural examinations which revealed *Staphylococcus* spp. as the most prevalent organisms (35.21%).

Sreedevi *et al.*, (2002) opined that in more than 80% of cases, the condition is limited to a single quarter involvement may be seen in very much lower percentage of cases, and there will be abnormal enlargement of udder and teats with gradual sloughing of the teats. The milk from the affected quarters was like custard, yellow in coloration and with clots or flakes.

Suarez *et al.*, (2002). Tests were associated to bacteriological analysis and classified into three groups: uninfected (negative culture), infected by minor pathogens and infected by major pathogens. Coagulase-negative *Staphylococcus* (32.4%), *Micrococcus* spp. (32.4%), *Corynebacterium* spp. (5.4%), and *Bacillus* spp. (1.4%) were the minor pathogens isolated, while *Staphylococcus aureus* (27%) and *Escherichia coli* (1.4%) were the major pathogens isolated. A good correlation was found between the CMT and SCC, which included inflammatory and epithelial cells (*r*=0.64; *P* < 0.0001). SCC averages for the CMT scores shown in parentheses were 223 576 (0); 245 248 (1); 397 778 (2); 1 159 109 (3) and 2 460 833 (4) cells/ml. The correlation between SCC and the infectious status of udder halves was 0.58 (*P* < 0.0001).

Dingwell *et al.*, (2003) conducted a study to evaluate the usefulness of the California mastitis test (CMT) to detect an intramammary infection caused by a major mastitis pathogen in early lactation cows. The gold standard used for comparison was bacteriological culture of single milk samples. Higher sensitivity (82.4%) and specificity (80.6%) of a positive CMT was observed on the 4th day of lactation.

Salaün *et al.*, (2004) The buffering capacity of milk products is an important physico-chemical characteristic that corresponds to the ability of the product to be acidified or alkalinized. The parameters of this value depend on several compositional factors including small constituents (inorganic
phosphate, citrate, organic acids) and milk proteins (caseins and whey proteins).

Bajwa et al., (2005) reported that mastitis quarter infused with 300 mg erythromycin at 12 hours interval for 5 days completely cured 73.53 per cent with significant improvement in quality of milk.

Das and Joseph (2005) 102 milk samples were collected from cases of clinical mastitis in buffaloes and 86 samples (84.31%) were found to be culturally positive for bacteria and yeasts.

Süheyla and Osman (2005) A total of 180 strains of Staphylococcus spp. were isolated from bovine mastitis, The isolates were identified as S. aureus (29.4%), S. hyicus (16.7%), S. intermedius (3.9%), S. chromogenes (16.1%), S. lentus (13.3%), S. epidermidis (11.1%), S. simulans (7.8%) and S. haemolyticus (1.7%). After growth, staphylococci were identified on the basis of colonycharacteristics, Gram staining, pigment production, hemolysis and the following biochemical reactions: catalase activity, coagulase test (rabbit plasma), oxidase test, O/F test with glucose, resistance to bacitracin (0.04 U), mannitol fermentation on Chapman Agar, urease, nitrate reduction, novobiocin resistance, phosphatase, deoxyribonucleose (DNase) test, carbohydrate (xylose, sucrose, trehalose, maltose, fructose, lactose, mannose) fermentation tests (8-10).

Atyabi et al., (2006)To determine the prevalence of bacterial mastitis in cattle, milk samples positive for California mastitis test were cultured. The bacterial species from 2904 milk samples studied were coagulase negative sp.in 879 (30.27%) samples, Streptococcus agalactiae in 642 (22.11%), S.dysagalactiae 332(11.43%), E.coli in 295(10.16%), Staphylococcus aureus in 84(2.89%), Bacillus cereus in 51(1.76%), Arcanobacterium pyogenes in31(1.07%), Pseudomonas aeruginosa in 6(0.21%), Klebsiella pneumoniae in 4(0.14%).

Detilleux et al., (2006) constructed a mathematical model of early response to Escherichia coli infection of the mammary gland. The model incorporates three equations that describe the interactions among bacteria, milk somatic cell count and blood leukocytes densities. The changes in milk cellular densities are mostly sensitive to variations in the rate of bacterial killing and in the rate of production of inflammatory cells.
Rainard and Riollet (2006) studied the interplay between the mammary gland innate defenses and mastitis causing bacteria, genetic dissection of immune response, microarray gene technology, transcriptomic methodologies and gene silencing by RNA interference which govern the susceptibility/resistance to mastitis at molecular and genetic levels.

Daimi et al., (2006) studied the therapeutic efficacy of Azadirachta indica bark and curcuma longa rhizome against subclinical mastitis and had seen the beneficial effect in cows.

Atyabi et al., (2006) reported that contamination of milk with coagulase negative staphylococci are most frequent bacterial infection in dairy cattle around Tehran, it mostly causes sub acute form of disease. S. agalactiae, S. dysgalactiae and Escherichia coli are second, third and fourth causative agents.

Awasthi and Upadhyay (2006) reported that mastitis was highest in crossbred cows (34.57 percent) followed by sahiwal cows (33.33 percent) and buffaloes (22.22 percent). Subclinical mastitis was found to be 33.88 percent. Right hind quarter (37.09 percent) affection followed by left hind quarter (27.74 percent) and left fore quarter (27.74 percent). Right fore quarter (19.35 percent) was least affected.

Reena Mukherjee et al., (2006) studied the effect of enrofloxacin and alfa tocopherol on the capacity of bovine milk leukocytes to generate reactive oxygen species and phagocytic activity after stimulation with phorbol 12–myristate 13-acetate (PMA) for superoxide in bovine clinical mastitis and showed that enrofloxacin and vitamin E have a beneficial effect on functioning of the immune cells in bovine udder affected by intramammary infection.

Roesch et al., (2006). One hundred fifty-eight isolates of quarter milk samples (93 from OP and 65 from IP cows) from quarters diagnosed with a CMT ≥2+ reaction were tested for antibiotic resistance. Of these, 79 isolates (46 from OP and 33 from IP cows) were identified as Staphylococcus aureus, 38 (19 OP, 19 IP) as nonaureus staphylococci, 28 (19 OP, 9 IP) as Streptococcus uberis, and 13 (9 OP, 4 IP) as Streptococcus dysgalactiae. The isolates were presumptively identified by Gram staining or potassium hydroxide tests. Staphylococci and streptococci were differentiated by
catalase activity. The *Staphylococci* were further identified based on coagulase activity, DNAse activity, and pigment production as *Staphylococcus aureus* or nonaureus *Staphylococcus* spp. The streptococci were identified to species level using Rapid ID 32 STREP (BioMérieux, Marcy l'Etoile, France) using cells grown anaerobically for 24 h at 37°C according to the instructions of the manufacturer.

Daimi *et al.*, (2006) reported that autogenous *staphylococcal* killed vaccine induces improvement in immunological parameters against subclinical mastitis in cows.

Moroni *et al.*, (2006) examined the antimicrobial susceptibility of 68 *Staphylococcus aureus* isolates from milk of cows affected by subclinical mastitis. The antimicrobial agents tested were the β-lactams, penicillin G, amoxicillin, ampicillin, cloxacillin, amoxicillin + clavulanate, cephalonium, and cefoperazone; and other drugs including lincomycin, oxytetracycline, doxycycline, and kanamycin. Minimum inhibitory concentrations recorded show that only certain β-lactamase–resistant penicillins (specifically cloxacillin) or penicillin combinations (amoxicillin + clavulanate) were consistently effective against *Staph. aureus*, whereas the other β-lactam derivatives and drugs from other pharmacological groups were either moderately effective or ineffective. Thus, β-lactamase–resistant penicillins are to be considered the antimicrobial agents of choice for treatment of bovine mastitis resulting from infection by *Staph. aureus*.

Vijayalakshmi *et al.*, (2007) reported *Staphylococcus aureus* as the main causal agent of mastitis, followed by *Streptococcus* spp. ciprofloxacin is the most effective drug against G+ve pathogen and enrofloxacin against G-ve. In unknown etiology, fluoroquinolones may be used for treating cases of bovine mastitis.

Nath and Dutta (2007) reported that incidence of subclinical mastitis was 73.46 percent cow wise and 73.82 percent quarter wise. The occurrence of disease were highest in animal of 3rd lactation (37.50 percent) followed by 4th lactation (20.83 percent) and second lactation (15.27 percent).
Nunes et al., (2007) conducted a study to evaluate the antimicrobial resistance traits of staphylococci responsible for subclinical bovine mastitis in Portugal, the minimum inhibitory concentrations (MIC) of 7 antimicrobial agents, frequently administered for mastitis treatment were determined for 30 Staphylococcus aureus and 31 Staphylococcus epidermidis field isolates. All Staph. aureus isolates showed susceptibility to oxacillin, cefazolin, gentamicin, sulphamethoxazole, trimethoprim, and enrofloxacin.

Patricia Medina and Larry Baresi (2007). Using TCA as the developing agent we have identified the hydrolysis of gelatin and casein within 3 h. When compared with conventional gelatin and casein hydrolysis techniques we have found the results of the TCA enhancement to be more rapid and sensitive than the conventional methods.

Sili and Vijaya Kumar (2007) studied 200 quarter milk samples collected from 50 crossbred cows. Out of which 68 quarters (34 percent) were found positive by CMT and on cultural examination only 39 were found positive from that Staphylococcus aureus, coagulase –ve Staphylococcus spp., Streptococcus agalactiae and Escherichia coli were isolated.

Singh et al., (2007) studied the effect of trisodium citrate in case of acute and sub acute mastitis and reported that there is increase in total protein, lactose, citrate and fat of affected quarter milk.

De and Mukherjee (2007) studied the clinical efficacy and antibacterial action of erythromycin against mastitis causing organism.

Ramprabhu and Rajeswar (2007) reported that among the indirect test, MCMT and mastrip showed 83.33 percent and 80.95 percent efficacy in diagnosing subclinical mastitis compared to cultural examination of the milk.

Munda et al., (2007) reported that bovine mastitis of coagulase positive staphylococci were sensitive to ciprofloxacin, cefotaxime and chloramphenicol. These strains were resistant to ampicillin, cloxacillin and roxithromycin. Coagulase negative strains were highly sensitive to cefotaxime.
followed by ciprofloxacin but comparatively resistant to lincomycin and tetracycline.

De and Mukherjee (2007) studied the cellular subpopulation in milk sample of healthy lactating cows and cows suffering from clinical mastitis. In animals suffering from clinical mastitis, neutrophils significantly increased to an extent of 375 percent, whereas lymphocytes reduced by 72 percent.

Reena Mukherjee (2007) studied antioxidant, anti-inflammatory and phagocytic activities of PMNs in milk isolated from healthy buffaloes and during mastitis with treatment of enrofloxacin alone and combination with enrofloxacin and vitamin E plus selenium. The results of the present experiment indicated enhancement of antioxidative and cellular defense and reduction of somatic cell count in the mastitic animal treated with enrofloxacin and vitamin E plus selenium as compared to enrofloxacin treatment alone.

Sahay et al., (2007) reported that Staphylococcus spp. is predominant organism for subclinical mastitis both in cows and buffaloes. Most of the isolates were highly sensitive to ciprofloxacin, enrofloxacin, pefloxacin and chloramphenicol and were least sensitive to ampicillin, oxytetracycline, cloxacillin and Kanamycin.

Tarfarosh et al., (2007) examined 450 bovine milk samples, Staphylococci were present in 72% to 76.3%, Streptococci in 12.5% to 14% and mixed infection in 9.7% to 11.2% of the samples tested. The periodic prevalence of Staphylococcal and Streptococcal mastitis and shift in the antibiograms of isolates over the period of these six years was recorded.

Hawari and Al-Dabbas (2008). Milk samples were collected from 220 lactating cows to determine the clinical and subclinical mastitis by white side test and confirmed by cultural tests. Staphylococcus aureus was considered to be the most common cause of both clinical and subclinical mastitis and followed by coliforms, streptococcus spp., corynebacterium spp., proteus spp. and pseudomonas spp. There results of sensitivity tests of the organisms isolated to antibiotics showed that 75% of strains were sensitive to Sulphamethaxazol Trimethoprim, 64% to Tetracycline, 54%
to Penicillin G, 52.8% to Ampicillin, 36% to Erythromycin and 21.4% to Neomycin.

Iqbal et al., (2008). 6522 milk samples from cattle, buffaloes, sheep and goats were tested for mastitis, out of which 1512 (23.18%) were found positive. Growth of different bacteria was yielded by 236 (15.16%) out of the positive ones. Gentamicin, enrofloxacin, norfloxacin and kanamycin were found most effective drugs amongst the 12 antibiotics tested in vitro.

Jakee et al., (2008): A total of 409 samples were investigated bacteriologically to detect the occurrence of *Staphylococci* among the diseased animals. A total of 78 S. aureus isolates secured from animals were characterized and identified using the most important conventional biochemical tests as anaerobic glucose fermentation, catalase, coagulase, Gelatin liquefaction, acetone production, novobiocin sensitivity and mannitol fermentation.

Saravanan et al., (2008) studied the efficacy of 10 different diagnostic tests to diagnose subclinical mastitis in bovines. Marathwada Agricultural University Mastitis (MAUM) test and Electrical conductivity test were found useful for detecting bovine subclinical mastitis. E.C. was superior to MAUM in identifying the etiological agent.

Vishnoi and Dang (2008) reported that percent change in milk neutrophils in conjunction with SCC may provide a more reliable method for assessing the quality of milk. Although the milk of mastitic buffaloes may appear normal after treatment, still their SCC remains very high and may take a longer time to stabilize.

Sood et al., (2008) reported that combination of somatic cell count (SCC) along with estimation of sodium and potassium electrolytes could be used as specific and sensitive markers for udder profile panel for early detection of subclinical mastitis.

Nam et al., (2008). Generally, gram-negative bacteria showed low susceptibilities to most of the antimicrobials tested in this study, except
amikacin and gentamicin. Although these 2 aminoglycosides were broadly active against gram-negative bacteria, less than half of those bacteria showed susceptibilities to streptomycin. The β-lactams, except piperacillin, had the lowest activity among antimicrobials tested in this study. Susceptibilities to chloramphenicol and trimethoprim were fairly high in all genera of gram-negative bacteria.

Palanivel et al., (2008) reported that prevalence of mastitis was higher in lactating than non-lactating buffaloes in early lactation stage than in mid lactation stage, summer season than in winter season and right hind quarter than any other quarter.

Shaheen et al., (2008) reported that animal suffering from clinical and subclinical mastitis had the concentration of milk malondialdehyde significantly higher (54.28 ± 2.1 nmol/ml) than healthy control (19.02 ± 1.7 nmol/ml), indicating alveolar cell damage during the process of mammary infection.

Ashire et al., (2008) reported that somatic cell count for udder secretions of dry buffaloes were very high ranging from $8.29 \times 10^5$ to $85.55 \times 10^5$ per ml of milk with mean value of $28.76 \times 10^5 \pm 0.94 \times 10^5$ per ml of milk.

Ashire et al., (2008) further they subjected for udder secretion for cultural examination from 24 buffaloes in dry period and 42 isolates were obtained. Among them, 17 were coagulase negative Staphylococci (40.47 percent), 7 Staphylococcus aureus (16.66 percent), 14 Streptococcus spp. (33.33 percent) and 4 Micrococcus spp. (9.52 percent).

Kheirabadi et al., (2008) reported that subclinical bovine mastitis in west Iran was mainly caused by coagulase positive Staphylococcus aureus and Streptococcus agalactiae.

La casse et al., (2008) studied the antibacterial and anti-inflammatory activities of lactoferrin protein in mammary gland of cow. Lactoferrin increases the inhibitory activity of penicillin in penicillin-resistant and penicillin-susceptible Staphylococcus aureus strain by repressing
transcription of β-lactamase gene. Bovine lactoferrin with penicillin-G is an effective combination for treatment of stable *Staphylococcus aureus* infections which are resistant to β-lactum antibiotics.

Kamboj *et al.*, (2008) reported that cattle with pendulous shaped, unbalanced and unduly large udder size were significantly more affected by subclinical mastitis (SCM) than the udder with cylindrical and lengthy treats.
MATERIALS AND METHODS

EXPERIMENTAL SPECIMENS: - Specimens for the work was collected as in table-1 below

<table>
<thead>
<tr>
<th>SOURCE OF SAMPLE</th>
<th>COW</th>
<th>BUFFALOES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>APPARENTLY HEALTHY</td>
<td>SUSPECTED ANIMALS</td>
</tr>
<tr>
<td>1. ORGANISED FARMS</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>-R.V.C farm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Kissan dairy farm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Military dairy farm</td>
<td></td>
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</tr>
<tr>
<td>2. PRIVATE FARM</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Dulia farm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Khatals</td>
<td>20</td>
<td>10</td>
</tr>
</tbody>
</table>
CLINICAL SPECIMENS:-

A total of 720 milk samples were collected from healthy as well from the suspected case of mastitis. Samples were collected by taking all aseptic precaution in sterile containers in 5-10 ml quantity. All samples were subjected for following tests:

1) Physical properties of milks
2) Mastect kit test
3) Methylene blue reduction test
4) Somatic cell count
5) California mastitis test
6) Isolation and identification of bacteria and fungi

1) Physical properties of milk
Physical properties of milk in respect to colour, consistency and pH were observed. Colour and consistency were observed visually but pH was measured by pH paper.

2) Mastect kit test
The kit was supplied by Indian immunologicals limited (Hyderabad). Separate strip was used for each quarter of the udder and first few strippings of milk was discarded. A drop of milk was taken on the strip and immediate colour change was observed. The colour change was compared with the colour index given in the inner side of booklet cover. The samples were graded as normal, suspected, sub-clinical and clinical as per direction of the firm.

3) Methylene blue reduction (MBR) test:-
For MBR test, 1 ml of methylene blue solution was added to 10 ml of milk. It was mixed properly and kept in a water bath. Control was prepared by taking 10 ml of milk in a test tube without adding methylene blue solution. The
tubes were examined at 30 min. interval. It was graded as per the ISI standard (1962).

   Grade I   – Very good – decolorised in 5 hours and above
   Grade II  – Good  – decolorised between 3-4 hours
   Grade III – Fair  – decolorised between 1-2 hours
   Grade IV  – Poor  – decolorised in 30 minutes

4) Somatic cell count (SCC):-

The method described by Chauhan et al., (2003) was used for somatic cell count. The collected milk was mixed thoroughly. An area of 1 cm² was marked in the center of a clean dry glass slide. 0.1 ml of milk sample was spread over the marked area with the help of bacteriological loop and a smear was prepared. Smear was air dried and fat was dissolved by addition of xylene and rinsing in tap water after 2-5 minutes. The smear was fixed with alcohol for 5 minutes and stained with methylene blue for 1 minute. Decolourisation was done with 95 per cent alcohol. The smear was observed under oil immersion for leucocytes count. The cell of 10 fields was counted and mean value multiplied by five lakhs to get the total number of leucocytes per ml of milk.

5) California mastitis test (CMT):-

California mastitis test was done as per the method of Suarez (2002). For the CMT approximately 1 ml of each quarter milk sample was taken on a plastic paddle marked left fore (LF), left hind (LH), right fore (RF) and right hind (RH). Equal quantity of CMT reagent was poured and mixed with milk sample by rotatory movement of the paddle. The change of the colour of reagent and the precipitation or gel formation was noted immediately for the degree of infection and the interpretation was made.
CMT score | Average SCC (cell per ml.) | Descriptions |
--- | --- | --- |
N (negative) | 3,00,000 | Nothing, homogenous |
1. | 9,00,000 | Distinct thick, no gel |
2. | 27,00,000 | Thick, immediately from gel |
3. | 81,00,000 | Gel is formed |

PROCEDURE FOR ISOLATION AND IDENTIFICATION OF BACTERIA AND FUNGI:-

1) MEDIA FOR ISOLATION AND IDENTIFICATION OF BACTERIA:-

For the isolation of bacteria various culture medias were used:-

i) Blood agar
ii) Nutrient agar
iii) Mac-conkey Agar
iv) Tryptose agar
v) EMB Agar
vi) Milk agar

CULTURE METHOD FOR ISOLATION OF BACTERIA:-

Isolation of bacteria was done as per the method of Cowan & Steel (1975). For the isolation of the bacteria the collected milk sample was incubated for a period of 8 hrs. Then a loopfull of milk sample was streaked on blood agar media. The plates were then incubated at 37°C for 24 hrs. After incubation the plates was examined for the presence of bacterial colony. All positive isolates was inoculated in different culture media for study of their cultural characteristics.
2) IDENTIFICATION OF ETIOLOGICAL AGENTS:-

i) Cultural characteristics

ii) Morphology and staining

iii) Biochemical profile:-

   a) Catalase test:-

      Catalase test was performed as per the method described by Thomas (1963). For this test culture was streaked on nutrient agar plates and incubated at 37°C for 24-72 hours. Catalase production was detected by adding 30% H₂O₂ over cultured plate after incubation. In positive cases gas bubbles were produced from the colonies.

   b) Coagulase test:-

      Coagulase test was performed as per the method described by Gillespie (1943). The test was performed to detect the presence an enzyme coagulase which caused clotting of rabbit plasma. For this test 0.5 ml of undiluted rabbit plasma was mixed with 0.1ml of 18 to 24 hours broth culture and incubated at 37°C for 1-6 hours. Diluted rabbit plasma without culture was used as control. The tubes were examined at 1, 3, and 6 hours interval for the development coagulum. Negative test tubes were left at room temperature overnight and then examined.

   c) Test for haemolysis:-

      For this test 5% sheep blood agar plates were used. Culture were streaked on the plates and incubated at 37°C. The plates were observed for any haemolytic activity up to 4 days.

   d) Pigment production test:-

      The pure culture was streaked on the surface of 33 per cent milk agar plate and incubated at 37°C for 24 hours followed by 48 hours at room temperature. Production of golden, yellow and white pigment was noted.
e) **Nitrate reduction:-**

The test strain was grown in 0.1 per cent nitrate broth and incubated for 5 days at 37°C. The presence of nitrite was tested by adding 1 ml of 0.8 per cent sulphansilic acid in 5 N acetic acid followed by 1 ml of 0.6 per cent diamethylalphanaphthylamine in 5 N-acetic acid. Appearance of red colour indicated the presence of nitrite.

f) **Carbohydrate fermentation test:-**

The method employed by Cowan and Steel (1970) was used. This test was based on the ability of bacteria to ferment carbohydrate and produce acid and gas. Peptone water containing one per cent sugar solution and bromothymol blue indicator was used for sugar fermentation test. Liquid paraffin was used for detection of gas production. Sugar tubes were inoculated with peptone water culture and incubated for 10 days at 37°C in positive sample tubes developed yellow colour and negative sample blue colour.

g) **Oxidation-fermentation test:-**

The method described by Hugh and Leifson (1953) was followed. Two tubes containing O-F media was taken, culture were stabbed in both the tubes with the help of straight bacteriological loop. One of the tubes were sealed with sterile liquid paraffin up to a depth of 1 cm. and both the tubes were incubated at 37°C and examined daily for 14 days. Yellow colour in one tube only indicated oxidation and no change in colour in both tubes indicated negative test.

h) **Gelatin liquefaction test:-**

The test was performed as per the method of Frazier (1926). This test was done to detect the ability of organism to produce enzyme gelatinase. For this 0.39 per cent gelatin agar plates were inoculated with culture and incubated at 37°C for 3 days. Post incubation the media were flooded with 12 per cent mercuric chloride solution. Clear zone was taken as positive for gelatin hydrolysis and opacity around the growth was considered as negative.
i) **Salt tolerance test:**

The test was performed by using 10% sodium chloride in nutrient broth media. The test strains were inoculated in above media and incubated at 37°C for 48 hours. Growth of the organism was observed in the media.

j) **Indole production test:**

The method as described by Cowan and Steel (1970) was used. The culture was inoculated in peptone water media and incubated at 37°C for 24 hours. After incubation 0.5 ml of Kovac's reagent was added to the media. Development of red/pink colour indicated positive test with production of indole. No change in colour indicated negative test.

k) **Methyl red and Voges proskauer test:**

The method by Cowan and Steel (1970) was followed. For this test cultures were inoculated in tubes containing glucose phosphate broth media and incubated at 37°C for 48 hours. After incubation each tubes was divided into two parts for the two tests separately. In one parts 2 drops of 0.04 per cent methyl red indicator was added. Red colour was indicative of positive reaction and yellow colour of negative reaction.

In another part 0.6 ml of 5 per cent alpha naphthol and 0.2 ml of 40 per cent KOH was added. Development of strong red colour in 15 minutes to 1 hour was indicative of positive reaction and development of no colour or slightly yellowish colour as negative reaction.

l) **Citrate test:**

Modified Koser's method (1923) was employed in this test. For this test Koser's Citrate media was used and culture was inoculated in this media. The tubes were then incubated at 37°C for more than 1 day. Development of blue colour from green after incubation indicated positive test.
2) MEDIA FOR ISOLATION OF FUNGI:-
   i) Saboraud’s dextrose Agar media with antibiotic
   ii) Saboraud’s dextrose Agar media without antibiotic

CULTURE METHOD FOR ISOLATION OF FUNGI:-

   Two sets of tubes containing SDA with antibiotic & without antibiotic were taken. Then a loop full of milk sample was inoculated in all 4 tubes. Two tubes, one containing antibiotic & other without antibiotic were taken from each set and incubated at 37°C for 30 days. The other two tubes were incubated at room temperature for 30 days. After incubation test tubes was examined daily for any visible growth. The tubes are discarded as negative only after observation upto 30 days.

2) IDENTIFICATION OF ETIOLOGICAL AGENTS:-
   i) Cultural characteristics
   ii) Morphology and staining
RESULTS

Present research was conducted for clinical and sub-clinical mastitis and the result has been concluded on the basis of isolation, identification, characterization and antibiotic sensitivity test of isolated organisms from suspected as well as apparently healthy cases of mastitis in cows and buffaloes kept in different farm conditions like organized farm, private farm and khatal. A total of 720 samples from all the four quarters of 110 cows and 70 buffaloes were collected for the present study.

All the milk samples were subjected to various physical examinations such as:

Variation in the consistency of milk samples:-

Variation in the consistency of milk due to bovine mastitis is presented in table-2. All 720 milk samples were examined for their consistency as- normal, serous, thin, thick and purulent. From the table it is evident that out of 440 samples collected from apparently healthy cows and buffaloes 279 samples of normal, 71 samples of thin and 90 samples were of thick consistency. On the other hand out of 280 samples collected from clinical cases of mastitis 204 samples of normal, 27 samples of thin and 25 samples were of thick consistency. Apart from this 8 samples of serous, 4 samples of purulent and 12 samples of blood mixed consistency were also found.

Variation in the pH of milk samples:-

pH values of the milk samples from cases of bovine mastitis caused by various isolates are given in table-03. Out of 440 samples collected from apparently healthy cows and buffaloes, 234 samples fell in the pH range of 6.5 to 7 and 206 samples fell in the range of 7.1 to 8. But none of the samples were found with pH value above 8. Out of the 280 samples collected from clinical cases of cows and buffaloes, 204 samples in the pH range of 6.5 to 7, and 68
samples in the pH range of 7.1 to 8 were found. Apart from this 6 samples of cows and 2 samples of buffaloes crossed the pH range of 8.

**Variation in the colour of milk samples:-**

Variation in the colour of milk due to bovine mastitis is presented in table-4. All 720 milk samples were examined for their colour as- White, cream, and blood tinged colour. From the tables it is evident that in case of 440 samples collected from apparently healthy cows and buffaloes, variation in colour from white (241 samples) to cream (199 samples) were observed. No sample of blood tinged colour was found. In case of 280 samples collected from clinical case of cows and buffaloes, wide variation in colour from white (200 samples) to cream (63 samples) were observed and 17 samples of blood mixed colour were also observed.

**Methylene blue reduction (MBR) test:-**

The rate of decolouration of milk due to bovine mastitis is presented in table-5. All 720 milk samples were graded as Grade I, Grade II, Grade III, Grade IV. Out of 440 samples collected from apparently healthy cows and buffaloes, 247 samples of grade I, 176 samples of grade II, and 17 samples of grade III were observed. But not even a single samples of grade IV was observed. But in case of 280 samples collected from clinical cases of cows and buffaloes, 202 samples of grade I, 10 samples of grade II, 27 samples of grade III and 41 samples of grade IV were observed.

**Somatic cell count (SCC):-**

Variation in the SCC of milk due to bovine mastitis is presented in table-6 and figure 1. All 720 milk samples were examined for their SCC which were recorded in four groups ie avg 3 lacs, avg 9 lacs, avg 27 lacs and more then avg 27 lacs. Out of 440 samples collected from apparently healthy cows and buffaloes, the
SCC of 230 samples (139 samples of cows and 91 samples of buffaloes) with an avg $<3 \times 10^5$, 171 samples (114 samples of cows and 57 samples of buffaloes) with an avg $9 \times 10^5$ and 39 samples (27 samples of cows and 12 samples of buffaloes) with an avg $27 \times 10^5$ were observed. No sample with an average SCC above 27 lacs was observed. But out of 280 milk samples collected from clinical cases of animals, the SCC of 204 samples (110 samples of cows and 94 samples of buffaloes) with an avg $<3 \times 10^5$, 08 samples (6 samples of cows and 2 samples of buffaloes) with an avg $9 \times 10^5$, 43 samples (25 samples of cows and 18 samples of buffaloes) with an avg $27 \times 10^5$ and 25 samples (19 samples of cows and 6 samples of buffaloes) with an avg $>27 \times 10^5$ were observed.

**California mastitis test (CMT):**

Variation in the CMT due to bovine mastitis is presented in table-7 and figure 2. All 720 milk samples were examined with CMT which were as- Normal, +, ++, ++++. Out of 440 samples collected from apparently healthy cows and buffaloes, 230 samples (139 of cows and 91 of buffaloes) were normal; 171 samples (114 of cows and 57 of buffaloes) were graded as (+) and 39 samples (27 of cows and 12 of buffaloes) were graded as (++) but none of the sample were of grade (+++). Out of 280 milk samples collected from clinical cases of animals, 204 samples (110 of cows and 94 of buffaloes) were normal, 08 samples (06 of cows and 02 of buffaloes) were graded as (+), 43 samples (25 of cows and 18 of buffaloes) were graded as (++) and 25 samples (19 of cows and 06 of buffaloes) of graded as (+++) were observed.

**Mastect kit test:**

The Variation in the colour of mastect kit test due to bovine mastitis is presented in table-8. All 720 milk samples were graded as normal, suspected, sub-clinical and clinical as per direction of the firm. Out of 440 samples collected from apparently healthy cows and buffaloes, 234 (143 samples of cows and 91
samples of buffaloes) were graded as normal, 131 (92 samples of cows and 39 samples of buffaloes) were graded as suspected and 75 (45 samples of cows and 30 samples of buffaloes) were graded as sub-clinical. But not even a single sample could be graded as clinical. But Out of 280 milk samples collected from clinical cases of animals 190 (106 samples of cows and 84 samples of buffaloes) were graded as normal, 15 (7 samples of cows and 10 samples of buffaloes) were graded as suspected and 75 (49 samples of cows and 26 samples of buffaloes) were graded as clinical. No sub-clinical case was detected in this group on the basis of mastect kit test.
ISOLATION OF ORGANISM:

All the collected milk samples were first streaked on 5 per cent sheep blood agar and incubated at 37°C for 4 days. The samples were examined daily. Single colony from each type of colony morphology was picked up and streaked on Nutrient agar, Tryptose agar, Mac-Conkey agar, EMB agar and Mannitol salt agar for their purification. The milk samples were also inoculated on SDA media with antibiotic and SDA media without antibiotic for the isolation of fungal agents (figure 3-7).

All the isolates obtained were grouped as the gm +ve cocci, gm -ve bacilli, gm +ve bacilli and yeast. Finally a total of 282 isolates were obtained. On the basis of gram and lacto phenol cotton blue staining and culture characteristics, all isolates were then grouped into four groups, 218 were gram positive cocci, 16 were gram positive bacilli, 44 were gram negative bacilli and 04 isolates were of yeast. (Table- 9 and 10)

On the basis of Physical properties of milk, MBR, Somatic cell count, California mastitis test, and cultural examination of milk, the samples were classified as clinical and sub-clinical. Where as based on Mastect kit test grouped them into normal, suspected, sub-clinical and clinical.

All 218 gram positive cocci isolates were reinoculated on suitable solid media and broth medium for morphological and cultural characterization and then subjected for biochemical test, as per method described by Cowan and Steel (1974) and were finally differentiated into 182 staphylococci spp, and 36 streptococci spp (figure 8,9,10).

All 44 gram negative bacilli were reinoculated on the Mac-conkey's agar plate and were further put on the eosin methylene blue (EMB) agar plate and then subjected for motility test, cultural characteristics and different biochemical tests as per standard method (Cruickshank et al, 1975 and Cowan and Steel (1974) and were differentiated into 26 E.coli spp, 12 Klebsiella spp, 06 Proteus spp (figure 11).

Similarly, 16 gram positive bacilli spp were recultured on suitable media and then subjected for morphological characterization and different biochemical
tests as per standard method (Cruickshank et al, 1975 and Cowan and Steel (1974).

The incidence of organism in different farms is presented in table 11. The result showed that in case of cows a significant difference exists between the three forms i.e the incidence was slightly higher (p<0.01) in Khatals (59.16%) then in private farms (46.66%) and organized farms (30%). In case of buffaloes also significant difference (P<0.05) was observed between the three farms i.e. Khatals (40.83%), Private farms (33.75%) organized farms (2.75%) as evident from chi-square test.

Chi-square test showed significant difference (P<0.01) in the number of positive cases between organized farms and private farms for cows. But in case of buffaloes non-significant different was observed in the number of positive cases between the 2 farms as shown in table 12.

In case of cows Chi-square test showed significant difference (P<0.05) for the incidence of organism between organized farms & Khatals, similarly in case of buffaloes also significant difference (P<0.01) was observed for the incidence of organism between the 2 farms as shown in table 13.

Chi-square test indicated that there was non-significant difference in incidence of organism between private farms & Khatals in case of both cows & buffaloes & the data have been illustrated in table 14.

Distribution of organisms between animals has been shown in table 15. Chi-square test indicated that incidence was slightly higher (P>0.01) in cows than in buffaloes (P>0.05).

The percent distribution of sub-clinical & clinical mastitis in cows & buffaloes has been presented in table 16. Significant difference (P>0.01) was observed between sub-clinical & clinical mastitis in case of cows & buffaloes.
Results of detailed examination of the tentatively groups Staphylococcus:-

All the 182 isolates tentatively grouped as Staphylococcus species were tested for coagulase production, pigment production, test for haemolysis, salt tolerance test, gelatin liquefaction and sugar fermentation test. (Table 17, 18)

126 isolates were coagulase positive, haemolytic, hydrolysed gelatin, produced 2 types of pigments i.e golden and yellow types, MR &VP positive, reduced nitrate to nitrite, and fermented sugars such as lactose glucose, sucrose, maltose, and , mannitol. These species were considered as Staphylococcus Aureus.

16 isolates were coagulase positive, haemolytic, hydrolysed gelatin, produced white pigmentation, MR positive, nitrate reduction positive, and fermented sugars such as lactose, glucose and sucrose but failed to ferment maltose, and , mannitol. These species were considered as Staphylococcus Intermedius.

20 isolates were coagulase negative, haemolytic, hydrolysed gelatin, produced white pigmentation, were positive for MR &VP test, reduced nitrate to nitrite, and fermented sugars such as lactose glucose, sucrose, maltose, and , mannitol and sensitive to novobiocin antibiotic. So, it was considered as Staphylococcus Epidermidis.

08 isolates were coagulase negative, non haemolytic with white pigmentation, hydrolysed gelatin, were positive for MR and nitrate reduction, and fermented sugars such as lactose glucose, maltose, and , mannitol except sucrose . These isolates were regarded as Staphylococcus Simulane.

12 isolates were coagulase negative, haemolytic producing white pigmentation, hydrolysed gelatin, , were positive for MR & nitrate reductions, and
fermented sugars such as lactose glucose, sucrose, maltose, and mannitol and they were resistant to novobiocin antibiotic and regarded as *Staphylococcus Saprophyticus*.

**Results of detailed examination of tentatively grouped Streptococcus:-**

All the 36 isolates which were tentatively grouped as streptococcus species were negative for Catalase test, nitrate reduction test, gelatin liquefaction and also failed to grow in 6.5% NaCl. They fermented sugars such as glucose, lactose, sucrose, salicine and trehalose. (Shown in Table-19)  

18 isolates which produced beta haemolysis on blood agar could not ferment sorbitol, mannitol and raffinose. They were regarded as *streptococcus agalactiae*.

13 isolates which produced narrow zone of haemolysis and fermented sorbitol but failed to ferment mannitol and raffinose were considered as *streptococcus dysgalactiae*.

05 isolates were non-haemolytic and fermented sorbitol and mannitol except raffinose. They were regarded as *Streptococcus uberis*. 
Results of detailed examination of tentatively grouped Corynebacterium

The 16 isolates which were tentatively grouped as *Corynebacterium* species produced small, dew drop like colonies on blood agar after 3 days of incubation.

The isolates were Gram positive rods, did not produce granular deposition like *streptococcus* in glucose phosphate broth, was catalase positive, nitrate negative, MR & VP negative, hydrolysed gelatin, produced beta haemolysis on blood agar and did not grow in 6.5% NaCl broth. They fermented sugars such as glucose, maltose and trehalose within 24 hours but did not ferment lactose, sucrose, salicine, mannitol, sorbitol, raffinose within 10 days. (shown in Table 20)

Results of detailed examination of tentatively grouped Gram negative bacilli:-

All the 44 gram negative rods tentatively grouped as enteric species were subjected to IMViC test, gelatin liquefaction, test for motility and sugar fermentation test. (Shown in Table-21)

26 isolates giving IMViC reaction +++-- which were motile, did not hydrolysed gelatin and fermented glucose, lactose, sorbitol, mannitol, sucrose, rhamnose but fail to ferment arabinose and adonitol. The isolates were regarded as *E. coli*.

12 isolates producing IMViC reaction -++++, which were non-motile, failed to hydrolysed gelatin and fermented sugars such as glucose (producing acid and gas), lactose, sorbitol, mannitol, sucrose, rhamnose, arabinose and adonitol. The strains were considered to be of Klebsiella Terrigengiving

06 isolates showing IMViC reaction ++++ which were motile, hydrolysed gelatin and fermented glucose (producing acid and gas), sucrose (producing acid) lactose, sorbitol, mannitol, rhamnose but fail to ferment arabinose and adonitol. The isolates were regarded as Proteus Vulgaris.
IN VITRO DRUG SENSITIVITY TEST

The antibiograms of different isolate were compared against the standard mentioned in table- 01a and results are depicted in the table 22, Figure 12. and graph-1-5. A total 282 isolates from clinical and sub- clinical cases of bovine mastitis were subjected to drug sensitivity test. In case of Coagulase positive staphylococcus the effectiveness of drugs were in the following order Gatifloxacin (76.05%), Levofloxacin (64.08%), Lomefloxacin (64.08%) and Ceftriaxone (54.92%). The organisms were moderately sensitive to Cloxacillin (49.29%), Gentamicin (48.59%) and Sparfloxacin (38.33%) but highly resistance to Ampicillin (35.91%), Fosformicin (34.50%) and Cotrimoxazole (23.23%).

In case of coagulase negative staphylococci the order of effectiveness of drug was Levofloxacin (82.50%), Gatifloxacin (77.50%) and Cloxacillin (77.50%), followed by Ceftriaxone (75.00%), Lomefloxacin (75.00%) and Gentamicin (70.00%). The organisms were moderately sensitive to Sparfloxacin (52.50%), Ampicillin (47.50%). but highly resistant to Fosformicin (40.00%) and Cotrimoxazole (32.50%).

Against the isolates of streptococci the order of drug effectiveness was Cloxacillin (72.22%), Sparfloxacin (69.44%), Ceftriaxone (69.44%), the organisms were moderately sensitive to Gentamicin (52.77%), Ampicillin (47.22%) and Fosformicin (33.33%) but highly resistant to Cotrimoxazole (13.88%).

Corynebacterium spp were highly sensitive to Gatifloxacin (87.50%), Ceftriaxone (81.25%), Levofloxacin (62.50%). The organisms were moderately sensitive to Sparfloxacin (56.25%), Lomefloxacin (56.25%) and Gentamicin (56.25%). but highly resistant to Cotrimoxazole (6.25%). Ampicillin (12.50%), Cloxacillin (25.00%), Fosformicin (18.75%).

Gram negative bacilli were highly sensitive to Cloxacillin (84.09%), Ceftriaxone (81.81%), Sparfloxacin (79.54%). followed by Levofloxacin (77.27%), Gatifloxacin (75.00%). The organisms were moderately sensitive to Lomefloxacin (72.72%) and Gentamicin (72.72%) and Fosformicin (36.36%). but highly resistant to Cotrimoxazole (9.09%). Ampicillin (18.18%).
The isolates of yeast showed complete resistance to all the antibiotics but when the isolates of yeast were tested against antifungal agents they showed sensitive to Nystatin (75%) and were resistance to Amphotericin B (25%).
DISCUSSION

Mastitis is a disease which affects the productivity of cattle & buffaloes to a great extent. It not only affects the productive & reproductive state of animal but leads to fibrosis of the udder which is of permanent nature, if not treated in time and animals become quite uneconomical.

The present study was undertaken to assess the incidence of mastitis in cattle & buffaloes under different farm conditions. It also included drug sensitivity tests to determine the resistance pattern of isolated organism in different mastitic milk samples. Firstly, the freshly collected milk samples were subjected to various physical examinations, like colour, consistency pH and various other tests such as CMT, SCC, MBR, Mastect kit test were also performed.

Effect on pH:

Generally the normal pH of cow milk ranges from 6.4 to 6.8. The acidity in milk is due to free casein, citrate, phosphate and dissolved carbon dioxide. Buffering capacity of milk is due to protein, citrate, phosphate, and bicarbonate as weak anions. (Salaün et al.,2004). Due to mastitis, there is increase in permeability which is followed by diffusion of milk with alkaline blood component mainly, bicarbonate, the mastitic milk has a higher pH, which is in accordance with the finding of Schalm et al. (1971).

Majority of the milk samples taken from apparently healthy animals were in the pH range of 6.5 to 7.0 and 7.5 to 8. But in case of samples collected from clinically affected animals, 6 of cows and 2 of buffaloes crossed the pH range of 8.

This suggested that there was a mild increase in pH of milk in various cases of mastitis. Higher rise in pH of milk samples were noted in cases of staphylococcal mastitis whereas as a moderate rise in pH in cases of streptococcal, E coli & Klebsiella mastitis.
Consistency of milk:

There was variation in consistency of milk samples, ranging from normal milky consistency to thin, thick serous, or purulent consistency with or without flakes. In few milk samples there was presence of blood in small proportion giving the milk blood tinged appearance. This change was found in milk samples which revealed isolation of organisms belonging to Staphylococcus, Streptococcus, Corynebacterium E.coli, Klebsiella, and Proteus species but not in samples from which yeast were isolated. In present study, majority of the milk samples had normal, thick or thin consistency and only few of the samples were of serous or purulent consistency. Thick consistency of milk was due to organisms such as Staphylococcus & Yeast. Flakes were observed mostly in samples with thin or serous consistency.

We can say that variation in consistency of milk other than those occurring normally during lactation, arise as a result of disease in general (Halversen etal.,1934). The findings are also similar to those of Schalm et al, (1971), who stated that mastitis exists in different forms with regard to etiological agent & pathogenesis. The variation in consistency of milk depends on the extent of inflammation. But the specific cause of mastitis could not be ruled out from consistency of milk.

So, we can say that the consistency of milk could not provide a clear picture about the isolates present in it. But the milk with thick or purulent consistency was invariably positive for Staphylococcus & Streptococcus species. Thin or serous consistency of milk was indicative of the increase in vascular permeability caused by various isolates of mastitis; which resulted in flow of plasma into milk. The presence of blood indicated haemorrhage due to damage of vascular endothelium.

In the present study milk samples collected from apparently healthy cows and buffaloes were of normal, thin, and thick consistency (with and without flakes) but none of the samples were found to be of serous, purulent and blood mixed consistency. But in case of samples collected from clinically affected animals along with normal, thin and thick consistency, few samples of serous, purulent and blood mixed consistency were also found.
Colour of milk;

The normal colour of milk ranges from yellowish creamy (cow milk) to creamy white (buffalo milk). The yellow colour of cow milk is due to the presence of carotene which imparts yellowish tint to milk.

In the present research work, the milk collected from various cases of mastitis were either white (61.25%), creamy (36.39%) or blood tinged (2.36%) in colour. These observations are in accordance with findings of Sreedevi et al., (2002).

The colour of milk was not found to be specific for a particular isolate. Blood mixed milk was found to be more in cases of Staphylococcus aureus followed by E.coli and other species of staphylococcus. But blood mixed milk was not a feature of yeast.

Somatic cell count:

The somatic cell count of mastitic milk sample was done as per the method described by Schalm et al (1971). Due to inflammation of mammary gland there is accumulation of leucocytes & epithelial cells i.e. neutrophils, lymphocytes & monocytes at the site of inflammation. So, the somatic cell count is the count of leucocytes & epithelial cells. The result is expressed as no. of somatic cells per ml of milk. Maximum acceptable limit of somatic cell count is 300000 per ml in case of normal milk in which majority constitutes of epithelial cells (50%), Schalm et al; (1971) and SUAREZ (2002). Change in milk neutrophils in conjunction with SCC may provide a more reliable method for assessing the quality of milk. Although after treatment the milk of mastitic buffaloes may appear normal, still their SCC remains very high and may take a longer time to stabilize, Vishnoi and Dang (2008).

In present study, high count of somatic cell was observed which was in highest range of (avg > 81 lacs/ml). Higher Somatic cell count was due to various isolates such as streptococcus, staphylococcus, E coli & yeast. This may be due to irritation caused to mammary parenchyma with infiltration of leucocytes at the site.
Some samples showed high somatic cell count but no bacterial isolates suggesting that the samples might be taken from animals after administration of antibiotics which inhibited the growth of organisms or required selective media for growth. Somatic cell count of less than 3 lacs per ml of milk was observed in higher percentage of milk samples, which suggested that the samples were collected from animals which were not suffering from bovine mastitis.

Enhanced somatic cell count was observed in case of bovine mastitis and has been reported by other workers such as Patricia Medina and Larry Baresi (2007), Soomro (2000). Our present study suggested that there was no direct correlation between consistency of milk & somatic cell count. This was clear from the fact that in case of mastitis arising from streptococcus species there was serous or thin consistency of milk still higher somatic cell count i.e. > 81 lacs/ml was observed. The present findings of higher specificity of SCC are online with Reena Mukherjee (2006) and SUAREZ (2002).

**California mastitis test:**

One of the best tests to detect mastitis is the California mastitis test. The animal body defends itself against injury and infection. When infection occurs, white blood cells, or leukocytes, gather to engulf bacteria and stop the spread of infection. If an injury is sterile, few leukocytes are present; but if infection sets in, the white blood cells congregate in great numbers.

High leukocyte counts in milk strongly indicated the presence of mastitis-causing bacteria. The CMT reagent is a detergent with a pH indicator added (reason for purplish color). When milk and CMT reagent were mixed in equal amounts, the CMT reagent dissolved or disrupted the outer cell wall and the nuclear cell wall of any leukocyte, which were primarily fat (detergent dissolves fat). DNA was then released from the nuclei which stringed together to form a gel. As the number of leucocytes increased in a quarter, the amount of gel formation also increased in a linear fashion. Higher reliability of CMT was indicated by SUAREZ(2002).
In the present study 720 quarters were screened by CMT, Out of which 282 quarters were found positive for mastitis. Samples taken from apparently healthy cases showed point score ranging from + weak positive (38.86%) to ++ distinct positive (8.86%). Whereas the samples taken from suspected cases of mastitis showed point score of all the 3 group, + weak positive (2.85%), ++ distinct positive (15.36%) and +++ strong positive (8.92%). The present findings of higher specificity of CMT is online with Dingwell et al; (2003) and Barbuddhi et al., (2001)

**Methylene blue reduction test:**

Methylene blue reduction test is based on the fact that the organisms present in milk are capable of reducing the blue colour of methylene blue to white with the help of enzyme reductase Schardinger [1902]. The removal of the oxygen from milk and the formation of reducing substances during bacterial metabolism cause the colour to disappear. The agencies responsible for the oxygen consumption are the bacteria. Though certain species of bacteria have considerably more influence than others, it is generally assumed that the greater the number of bacteria in milk, the quicker will the oxygen be consumed, and in turn the sooner will the color disappear. Thus, the time of reduction is taken as a measure of the number of organisms in milk.

In the present study, the milk samples were divided into four grades according to M.B.R test. The samples taken from apparently healthy animals were mostly of grade I and grade II. But the samples taken from suspected cases of mastitis were of grade III and grade IV which indicated poor quality of milk in latter case. The present findings are online to those of Atherton and Newlander (1977).

**Mastect kit test:**

Mastect is a booklet of bromothymol blue impregnated strips of paper. It is a very unique and simplest aid developed by the National Dairy...
Development Board, Anand and manufactured by Indian Immunologicals Limited (Hyderabad) for the detection of mastitis in dairy animals under field conditions. Mastect kit test seems to be a very important tool for detection of sub-clinical mastitis because generally cultural examination cannot be performed in the field.

In case of Mastect kit test the change in colour of milk samples indicated the state of mastitis that is: Yellow colour indicating normal, yellowish green- suspected, green- sub- clinical and blue- clinical mastitis. The suspected samples could be further graded as normal or sub-clinical after cultural examinations. In our present research the samples taken from apparently healthy animals were graded as normal, suspected and sub-clinical mastitis. But the samples taken from suspected cases were mostly clinical, which was indicated by the change in colour from yellow to blue.

Results of Mastect kit test cannot be compared with the findings of other workers due to non-availability of literature on its use.

**Microbiological study**

For the purpose of primary isolation of organisms 5% sheep blood agar was used. It supported the growth of all kinds of organisms which were the causative agents of mastitis. It provided an opportunity to determine their haemolytic activity. Other selective medias like MacConkey agar media, EMB agar media, Tryptose agar media etc were not used for primary isolation because of their selective nature when used as selective media. In order to prevent the contamination of mastitic milk from other organisms, only fresh milk samples were used for streaking on the plates.

Coagulase test is the most commonly used method for the identification of pathogenic staphylococci & is the criterion generally accepted for membership of species Staphylococcus aureus (William smith 1947). This property as an index of pathogenicity was indirectly supported by Raymond et al., (1970) who considered the absence of coagulase production as a strong indicator of non pathogenicity. But Barbar (1960) regarded positive coagulase test as only a rough guide and not an indicator of full pathogenicity & virulence.
The method of Goldstein (1982) has been adopted for performing coagulase test. The use of rabbit/human plasma in coagulase test has been advocated by Hwang (1997), Harry (1972) & other investigators.

In this case rabbit plasma has been used. Tube method was adopted in which 1:5 dilution of citrated rabbit plasma was used. Out of total 182 isolates of Staphylococcus species, 142 isolates were coagulase positive & 40 isolates were coagulase negative. Out of the 142 coagulase positive strains, 126 strains were of Staphylococcus aureus and 16 strains were of S. intermedius. Earlier classification was based on pigment production, the degree of pigmentation also varies according to medium used & condition of incubation, Süheyla et al., (2005). Milk agar was used for the study of pigment production (Christie & Keogh (1940) and the same was used in the present study.

Cowan (1938) found 8 out of 13 coagulase positive strains to produce golden pigment and 5 white, 3 of 7 coagulase negative strain were golden, 3 white and 1 yellow. William Smith (1947) found 79 of 110 pathogenic animal strains to be golden, 29 white and 2 yellow, 17 of 63 non pathogenic strains produced golden pigment, 44 white and 2 yellow. Roesch et al.,(2006) also advocated that pigment formation was not stable characteristic of staphylococci. In our present study out of total 142 coagulase positive strains of staphylococcus 82 produced golden pigment, 44, yellow and 16 white pigments. Among 40 coagulase negative strains, 8 produced white pigment and were of non-haemolytic whereas 32 were of haemolytic strain, Thus we can say that pigment production is not only a property of coagulase positive strains but also of coagulase negative strains. So, classification of staphylococci should not be done on the basis of pigment production. (William Smith (1947), Süheyyla et al., (2005), and Roesch et al.,(2006).

Gelatin liquefaction was earlier considered to be one of the important characteristic of Staphylococcus aureus. Joshi et al., (1963) found those 106 out of 110 pathogenic strains of animals and 35 of 63 non pathogenic strains liquefied gelatin. Süheyyla et al., (2005) also had similar observation and reported about 93% of gelatinase producing strains isolated from samples of
bovine mastitis. But the observations of Jakee et.al.,(2008): were quite different, as only 50% of their enterotoxin positive and negative strains liquefied gelatin. Eleck & Levy (1950) observed that out of 195 coagulase positive strains only 63% strains were gelatin liquefiers. Das et al., (2005) described this property not useful for differentiating Staphylococcus from milk. In the present study all the 142 coagulase positive strains and 40 coagulase negative strains liquefied gelatin. Thus, for pathogenic strains of animal origin, gelatin liquefaction may not be considered as an important characteristic.

Sugar fermentation test for Staphylococcus aureus was not found to be of much differential value in accordance with the observation of other workers; there was production of acid but no gas from sugars such as sucrose, lactose, maltose, glucose and mannitol. The production of acid from mannitol was considered as an important property of pathogenic strains which is not true for animal Joshi et al., (1963). Mannitol fermentation which was considered as an important criterion for indication of pathogenicity of Staphylococcus aureus is not fairly true. The results of present study also indicated that both coagulase positive and coagulase negative strains were positive for mannitol fermentation.

Streptococci have been mainly studied with regard to their hemolytic properties. This property is most valuable tool and was studied using blood agar media which supported the growth of many Streptococcus species. Other features for identification of Streptococci are morphology, fermenting ability of carbohydrates & other substances, heat resistance & high salt tolerance property.

Zemelman. and Longeri (1964)). described the appearance of various Streptococci on blood agar. He classified different types of hemolysis as Beta, Alpha prime, Alpha & Gamma. The use of first three Greek letters Alpha, Beta, Gamma as designation was suggested by Rao et al.,(1989) who reported that under certain conditions streptococci belonging to different viridians groups’ causes hemolysis of blood cells.

The alpha group is the viridians group which includes those causing an alteration of haemoglobin without freeing it from cells, in its course its colour is changed through various shades of green to greenish black. The Beta type includes those forming a soluble haemolysis that causes freeing of
haemoglobin from erythrocytes and is the haemolytic group and third group is the Gamma type, non-haemolytic group which includes those causing no noticeable change in erythrocytes. In the present study 18 of 36 strains of Streptococci produced Beta type of haemolysis, 13 Alpha type and 05 were of non-haemolytic types.

Corynebacterium species has been recovered from cattle suffering from different diseases such as endometritis, pyometra, liver, abscess, polyarthritis, nephritis etc. It has also been reported from lesions of summer mastitis during months of July, August & September (Watts and Rossbach .,(2000). In the present study 16 Corynebacterium species were isolated from mastitic milk samples.

On blood agar, characteristic behaviour of the organism was noted, no growth was observed after 24 hours of incubation at 37c. Only after 36 hour, full size growth and after 48 hour of incubation narrow zone of haemolysis (Beta haemolysis) was observed. The strains hydrolyzed gelatin and arginine and also fermented glucose without gas and acid and gas from lactose, maltose, and trehallose Zemelman and Longeri. (1964)).

Chronic forms of mastitis associated with the bacteria of the Friedlanders group (Klebsiella) have been described by Atyabi (2006). Various workers had reported variation in M.R and V.P tests. In present work, M.R negative and V.P positive strains were observed. The strains were also indole negative and non-motile which were in accordance with the finding of Atyabi (2006).

Isolation and identification of 6 proteus spp. was done on the basis of their characteristic swarming colonies on nutrient and blood agar plates .they were motile and non lactose fermenter.

In this study a total of 720 milk samples collected from all the four quarters of 110 cows (70 apparently healthy and 40 clinically affected) and 70 buffaloes (40 apparently healthy and 30 clinically affected) were examined for bacterial isolations. Among 282 isolates, 64.54% isolates Staphylococcus species. And 12.77% were of Streptococcus spp. the above finding is similar to the findings of Tarfarosh et al., (2007) examined 450 bovine milk samples. Staphylococcus was found to be 72% to 76.3%, and Streptococcus in 12.5% to 14%.
Observations on the isolation of Staphylococcus made by other workers are also in accordance with our results. [Pal et al ;(1988)53%, Singh et al; (1994)73.68%, Wadhwa et al;(1996)68.83% and Banerjee et al (2002)54.85%] The percentage of coagulase positive staphylococcus species was 50.53% and coagulase negative staphylococcus species was 14.18% which is similar to Shriram et al(1997)58.33% coagulase positive staphylococcus species and Rahman et al ;(1982) 41.17% and Banerjee et al;(2002) 61.18% coagulase negative staphylococcus species.

From the total 720 samples taken from both apparently healthy and clinical cases of mastitis streptococci were isolated from 36(5%) of the samples. According to Shukla et al ;(1998)31.98%, Kothe et al (1993)27.67% and Chowdhary et al (2002)23.08% streptococcus species were isolated. The results indicate lower number of Streptococci than Staphylococci which may be due to the unsurviveability of Streptococci invtro for longer period (Schalm et al, 1971).

About 5.67% of 282 isolates belonged to Corynebacterium species isolates, This is similar to findings of Chowdhary et al; (1982),9.82%,Rahman et al(1984)3.4%, Shukla et al;(1998).

Of 16 total isolates of Corynebacterium, 8 were isolated from sub clinical mastitis and 8 from clinical cases. It appears that Corynebacterium spp. allows other pathogen to cause acute disease resulting into mixed infection (Singh et al; 1990 and Ramchandraiah et al; (1990).

12 isolates were of Klebsiella out of the 282 isolates. This finding is similar to Rehman et al; (1984),Shukla et al;(1998) and Chowdhary et al;(2003).

Only 2.13% Isolates were observed as Proteus spp., which is on line with reports of Gupta et al ;(1982)3.35%, Wadhwa et al;(1996)Shukla et al;(1998) and Rahman et al;(1982)4.65%.

From the results obtained we can conclude that total of 180 animals constituting 70 clinical and 10 from apparently healthy animals were screened, yielded Staphylococcus spp, Streptococcus spp, Corynebacterium spp, Klebsiella spp, Proteus spp and yeast.
In vitro drug sensitivity test:-

The results of drug sensitivity test are given in table 15. All the 282 isolates (278 bacterial and 4 yeast) from clinical and sub clinical cases of bovine mastitis were subjected to drug sensitivity test. The test revealed that coagulase positive Staphylococcus were highly sensitive to Gatifloxacin(76.05%), Levofloxacin(64.08%), Lomefloxacin(64.03%) and Ceftriaxone(54.92%) which is online to the finding of Gupta et al., (1982) and moderate sensitivity was recorded for Cloxacillin (49.29%), Gentamicin (48.59%) and Sparfloxacin (38.33%) which is similar to the findings of Nunes et al.,(2007) and Moroni et al.,(2006) but high resistance was observed for drugs such as Ampicillin (35.91%), Fosformicin (34.50%) and Cotrimoxazole (23.23%) which is similar to those of Madhu Babu et al; (1993),and Bhattacharya et al.,(2002).

The coagulase negative isolates showed sensitivity pattern similar to those of coagulase positive isolates but were moderately sensitive to Sparfloxacin (52.50%), and Ampicillin (47.50%). While showed considerable resistance to Fosformicin (40.00%) and Cotrimoxazole (32.50%) which is in accordance to Munda et al (2007). Thus Staphylococcus species were sensitive to almost all the antibiotics except Ampicillin and Cotrimoxazole.

Drug sensitivity pattern for streptococcus species showed higher sensitivity to Cloxacillin (72.22%), Ceftriaxone (69.44%), and Sparfloxacin (69.44%) but were moderately sensitive to Gentamicin (52.77%), Ampicillin (47.22%) and Fosformicin (33.33%) which is online to the findings of Iqbal et al; (2008).High resistance pattern of the organism to Cotrimoxazole (13.88%) is in accordance with the finding of Bhattacharya et al.,(2002).

*Corynebacterium* spp were highly sensitive to Gatifloxacin (87.50%), Ceftriaxone (81.25%) and Levofloxacin (62.50%) but moderately sensitive to Sparfloxacin (56.25%), Lomefloxacin (56.25%) and Gentamicin (56.25%).These findings are in accordance with those of Rao et al.,(1989), Hawari et al., (2008).The isolates were highly resistant to Cotrimoxazole (6.25%), Ampicillin (12.50%), Cloxacillin (25.00%) and Fosformicin (18.75%) which is online with Jae-Choon et al.,(2000)
Gram negative bacilli were highly sensitive to Cloxacillin (84.09%), Ceftriaxone (81.81%), Sparfloxacin (79.54%) followed by Levofloxacin (77.27%), Gatifloxacin (75.00%). The organisms were moderately sensitive to Lomefloxacin (72.72%) and Gentamicin (72.72%) and Fosformicin (36.36%) which is online to the finding of Nam et al., (2008) but highly resistant to Cotrimoxazole (9.09%) and Ampicillin (18.18%). The finding is similar to Pal et al.; (1988), Bhattacharya et al., (2002).

The 4 isolates of yeast showed complete resistance to all the antibiotics. The may be due to the fact that yeast has got thick wall with starch and antibiotics have got no effect on it. This finding is on line with the finding of Chung Shih Te; et al.(2002). But when the isolates of yeast were tested against antifungal agents they showed sensitive to Nystatin and were resistance to Amphotericin B. Similar resistance pattern been observed by other workers as well Pianta (1995).

In a nutshell 110 cows and 70 buffaloes were screened for clinical and sub-clinical mastitis by various physical examinations, cultural isolation & identification and drug sensitivity tests performed on all the 282 isolates obtained from 720 milk samples. The milk samples were also subjected to other tests such as California mastitis test, Somatic cell count, Methylene blue reduction test, Mastect kit test etc and the result was noted. The study revealed that the isolates of Staphylococcus species was highest followed by Streptococcus, Gram negative bacilli, and Corynebacterium.

The study led to the fact that in Ranchi district of Jharkhand the most common organism causing mastitis was found to be Staphylococcus aureus. The incidence of clinical and sub-clinical mastitis in cows was higher i.e. 31.25% and 48.92% than in buffaloes i.e. 21.67% and 43.13% respectively. Buffaloes were comparatively resistant to mastitis than cows which might be due to the presence of thick and compact epithelium, thick keratin layer and better organised sphincter muscles of teat canal providing resistance to buffaloes. The prevalence of mastitis was lower in organized farms, slightly higher in private farms and highest in khatals. Various tests
such as Mastect kit test can be used for detection of sub-clinical mastitis using milk samples in farm itself and drug sensitivity test was performed to find out the sensitivity of organism to a particular drug, which revealed that against majority of Staphylococcus isolates Gatifloxacin (76.05%) was effective, followed by Cloxacillin (72.22%) against Streptococcus. This will help not only in removing the organisms causing mastitis but also in gaining the normal milk production. So with better managerial practices and strict monitoring and surveillance the incidence of mastitis can be lowered to a greater extent.
The present study was undertaken to investigate the incidence of clinical and sub-clinical mastitis in cows and buffaloes under different farm condition. For this study a total of 720 milk samples were collected of which 280 samples were collected from 70 buffaloes and 440 samples were collected from 110 cows. Out of 280 samples taken from buffaloes, 120 samples were from clinically affected and 160 samples were from apparently healthy animals. Similarly, out of 440 samples collected from cows, 160 samples were from clinically affected and 280 samples were from apparently healthy animals. All the samples were procured from organized farms, private farms and khatals of Ranchi.

The overall incidence of clinical and sub-clinical mastitis in cows were higher i.e. 31.25% and 48.92% as compared to buffaloes i.e. 21.67% and 43.13% respectively.

Out of 720 samples collected from cows and buffaloes 282 samples were considered as positive for mastitis. A total of 282 bacterial isolates were found of which 182 strains were of Staphylococcus species comprising of 142 coagulase positive and 40 coagulase negative species, 36 Streptococcus species, 16 corynebacterium species, 26 E. coli. 12 klebsiella species, 06 proteus and 04 were of yeast.

The prevalence of organism in cows and buffaloes was lower in organized farms (30%, 23.75%), slightly higher in Private farms (46.66%, 33.75%) and highest in khatals (59.16%, 40.83%) respectively.

All 282 bacterial isolates were subjected to in vitro drug sensitivity tests using different antibiotics disc procured from “ Pathoteq biological laboratories, India “. The drug sensitivity test showed that staphylococcus species were highly sensitive to Gatifloxacin , Levofloxacin, Lomefloxacin and Ceftriaxone . The organisms were moderately sensitive to Cloxacillin , Gentamicin and Sparfloxacin but highly resistance to Ampicillin, Fosformicin and Cotrimoxazole .
In case of coagulase negative staphylococci the order of effectiveness of drug was Levofloxacin, Gatifloxacin and Cloxacillin, followed by Ceftriaxone, Lomefloxacin and Gentamicin. The organisms were moderately sensitive to Sparfloxacin, Ampicillin, but highly resistant to Fosformicin and Cotrimoxazole.

Against streptococci isolates the order of drug effectiveness was Cloxacillin, Ceftriaxone followed by Gatifloxacin, Levofloxacin. The organisms were moderately sensitive to Gentamicin, Ampicillin, and Fosformicin but highly resistant to Cotrimoxazole.

*Corynebacterium* spp were highly sensitive to Gatifloxacin, Ceftriaxone, Levofloxacin. The organisms were moderately sensitive to Sparfloxacin, Lomefloxacin and Gentamicin, but highly resistant to Cotrimoxazole. Ampicillin. Cloxacillin, Fosformicin.

Gram negative bacilli were highly sensitive to Cloxacillin, Ceftriaxone, Sparfloxacin, followed by Levofloxacin, Gatifloxacin. The organisms were moderately sensitive to Lomefloxacin, Gentamicin and Fosformicin, but highly resistant to Cotrimoxazole. Ampicillin.
1. Incidence of sub-clinical mastitis was higher as compared to clinical mastitis in both cows and buffaloes under all farm conditions.
2. The incidence of mastitis was recorded higher in cows (42.50%) than in buffaloes (33.92%).
3. The overall incidence of clinical and sub-clinical mastitis in cows were higher i.e. 31.25% and 48.92% as compared to these in buffaloes i.e. 21.67% and 43.13% respectively.
4. The prevalence of organism in cows and buffaloes was lower in organized farms (30%, 23.75%), slightly higher in Private farms (46.66%, 33.75%) and highest in khatals (59.16%, 40.83%), which might due to strict hygienic measures adopted in organized farms during and after milking.
5. Drug sensitivity tests revealed the overall effectiveness of Gatifloxacin and levofloxacin against almost isolates and Ceftriazone in case of grams negative organism were highly effective drug.
6. Strict monitoring and surveillance measures in case of mastitis, drug sensitivity tests of organism causing mastitis and proper hygienic measures may be carried out in our state for the control of mastitis.


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Antibiotic sensitivity test

In vitro drug sensitivity of selected representative isolates was done as per method described by Cruickshank et al (1975). For streptococci serum agar plates and for all other bacteria the nutrient agar plates were taken. A 0.1 ml broth culture was poured and spread over the whole surface with the help of sterile L-spreaders. Commercial antibiotic discs were placed on the inoculated agar plates with the help of sterile forceps and pressed gently. Then plates were incubated at 37°C for 24 hours. Zone of inhibition was measured with the help of slide calipers. Interpretations were done on the basis of zone size as sensitive (s), resistant(R), intermediate (I) as in Table-1a below.

<table>
<thead>
<tr>
<th>Antibiotic or Chemotherapeutic agent</th>
<th>Symbol</th>
<th>Strength</th>
<th>Resistant mm or less</th>
<th>Intermediate mm</th>
<th>Sensitive mm or more</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gatifloxacin</td>
<td>GT</td>
<td>5 mcg</td>
<td>14</td>
<td>15-17</td>
<td>18</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>SC</td>
<td>5 mcg</td>
<td>15</td>
<td>16-18</td>
<td>19</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>CX</td>
<td>1 mcg</td>
<td>11</td>
<td>12-13</td>
<td>14</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>CT</td>
<td>30 mcg</td>
<td>13</td>
<td>14-20</td>
<td>21</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>FO</td>
<td>200 mcg</td>
<td>12</td>
<td>13-15</td>
<td>16</td>
</tr>
<tr>
<td>Lomefloxacin</td>
<td>LO</td>
<td>10 mcg</td>
<td>18</td>
<td>19-21</td>
<td>22</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>LA</td>
<td>5 mcg</td>
<td>15</td>
<td>16-18</td>
<td>19</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>GM</td>
<td>10 mcg</td>
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<td>13-14</td>
<td>15</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>AS</td>
<td>10 mcg</td>
<td>13</td>
<td>14-16</td>
<td>17</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>BA</td>
<td>25 mcg</td>
<td>10</td>
<td>11-15</td>
<td>17</td>
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<tr>
<td>Cases</td>
<td>Animals</td>
<td>Diff. farm conditions</td>
<td>Normal</td>
<td>Serous</td>
<td>Thin</td>
</tr>
<tr>
<td>-------</td>
<td>---------</td>
<td>----------------------</td>
<td>--------</td>
<td>--------</td>
<td>------</td>
</tr>
<tr>
<td>36</td>
<td>10</td>
<td>Private farm</td>
<td>10</td>
<td>06</td>
<td>06</td>
</tr>
<tr>
<td>55.00</td>
<td>13</td>
<td>Khatal</td>
<td>10</td>
<td>04</td>
<td>02</td>
</tr>
<tr>
<td>20.00</td>
<td>15</td>
<td>Clinical Cow</td>
<td>10</td>
<td>02</td>
<td>07</td>
</tr>
<tr>
<td>77.50</td>
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<td>Clinical Private</td>
<td>10</td>
<td>02</td>
<td>07</td>
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<td>18.50</td>
<td>10</td>
<td>Clinical Khatal</td>
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<td>02</td>
<td>07</td>
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<td>26.00</td>
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<td>Apparently Healthy Cow</td>
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<td>02</td>
<td>07</td>
</tr>
<tr>
<td>31.00</td>
<td>14</td>
<td>Apparently Healthy Buffalo</td>
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<td>02</td>
<td>07</td>
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</table>

Table 2: Variation in the consistancy of milk sample
### Table 3. Variation in pH of milk sample

<table>
<thead>
<tr>
<th>PH Value</th>
<th>Apparently Healthy Cases</th>
<th>Clinical Cow Cases</th>
<th>Buffalo Cases</th>
</tr>
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<tbody>
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<td>7.1-8</td>
<td>20</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>7-7.1</td>
<td>20</td>
<td>30</td>
<td>10</td>
</tr>
<tr>
<td>&lt;7</td>
<td>20</td>
<td>30</td>
<td>10</td>
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</table>

<table>
<thead>
<tr>
<th>Animals</th>
<th>Diff. farm conditions</th>
<th>Total animals</th>
<th>PH Value</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow Organised</td>
<td></td>
<td>30</td>
<td>6.5-7</td>
<td>20</td>
<td>66.67</td>
</tr>
<tr>
<td>Private Farm</td>
<td></td>
<td>47</td>
<td>6.5-7</td>
<td>20</td>
<td>66.67</td>
</tr>
<tr>
<td>Khatal</td>
<td></td>
<td>30</td>
<td>6.5-7</td>
<td>20</td>
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</tr>
<tr>
<td>Buffalo</td>
<td>Private Farm</td>
<td>80</td>
<td>6.5-7</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Organised</td>
<td></td>
<td>47</td>
<td>6.5-7</td>
<td>20</td>
<td>66.67</td>
</tr>
<tr>
<td>Private Farm</td>
<td></td>
<td>30</td>
<td>6.5-7</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>Khatal</td>
<td></td>
<td>66.67</td>
<td>6.5-7</td>
<td>20</td>
<td>66.67</td>
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</table>
### Table 4: Variation in the colour of milk sample

<table>
<thead>
<tr>
<th>Cases</th>
<th>Animals</th>
<th>Diff. farm conditions</th>
<th>Total number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Number</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>White colour</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cream colour</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Blood tinged colour</td>
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</tr>
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### Table 5. Variations in Methylene blue reduction test of Milk Samples

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<tr>
<th>MBR</th>
<th>Grade-I</th>
<th>Grade-II</th>
<th>Grade-III</th>
<th>Grade-IV</th>
<th>Total Animals</th>
<th>Diff. Farm Conditions</th>
<th>Animals</th>
<th>Cases</th>
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<tr>
<td>03</td>
<td>04</td>
<td>02</td>
<td>00</td>
<td>10</td>
<td>Organised Farm</td>
<td>Khatal</td>
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<td>Cow</td>
</tr>
<tr>
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<td>04</td>
<td>02</td>
<td>00</td>
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<td>05</td>
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<td>01</td>
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<td>Cow</td>
</tr>
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<td>02</td>
<td>01</td>
<td>20</td>
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<td>-</td>
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<td>10</td>
<td>Private Farm</td>
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<td>-</td>
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<td>30</td>
<td>10</td>
<td>Private Farm</td>
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<td>-</td>
<td>05</td>
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<td>20</td>
<td>Private Farm</td>
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<tr>
<td>-</td>
<td>05</td>
<td>38</td>
<td>37</td>
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<td>Private Farm</td>
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<tr>
<td>-</td>
<td>03</td>
<td>40</td>
<td>30</td>
<td>30</td>
<td>Private Farm</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Cases: Apparently healthy Cow

Clinical Cow

Khatal

Private Farm

Organised Farm

Buffalo
<table>
<thead>
<tr>
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Table 7. Variations in California Mastitis Test
### Table 8: Variations in Mastect Kit Test of Milk Samples

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**Legend:**
- Blue (clinical)
- Green (sub clinical)
- Yellowish (suspected)
- Yellow (Normal)
- Khatal
- Private farm
- Organised farm
- Clinical
- Apparently healthy
- Cases
- Total animals
- Diff. farm conditions
- Animals
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Table 9. Organisms isolated fromApparently healthy Milk and Suspected Milk Samples
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<td>Total No. of Isolates</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Staphylococcus spp.</td>
<td>100</td>
<td>440</td>
</tr>
<tr>
<td>2</td>
<td>Streptococcus spp.</td>
<td>14.47</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>Corynebacterium</td>
<td>10.53</td>
<td>88</td>
</tr>
<tr>
<td>4</td>
<td>Gram –ve bacilli</td>
<td>14.47</td>
<td>10.53</td>
</tr>
<tr>
<td>5</td>
<td>Yeast</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Source of sample</td>
<td>No. of sample</td>
<td>No. of positive case</td>
<td>Percentage</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------</td>
<td>----------------------</td>
<td>------------</td>
</tr>
<tr>
<td>Cows</td>
<td>200</td>
<td>60</td>
<td>30%</td>
</tr>
<tr>
<td>Buffaloes</td>
<td>280</td>
<td>80</td>
<td>28.4%</td>
</tr>
<tr>
<td>Khatals</td>
<td>120</td>
<td>49</td>
<td>40.83%</td>
</tr>
<tr>
<td>Private farms</td>
<td>120</td>
<td>71</td>
<td>59.16%</td>
</tr>
<tr>
<td>Organized farms</td>
<td>200</td>
<td>80</td>
<td>40.83%</td>
</tr>
<tr>
<td>Total</td>
<td>440</td>
<td>187</td>
<td>42.50%</td>
</tr>
</tbody>
</table>

** = P< 0.01  
*=P<0.05

Table-11. Incidence of organism in different farms.
<table>
<thead>
<tr>
<th>Source of sample</th>
<th>Cows</th>
<th>Chi-square</th>
<th>Buffaloes</th>
<th>Chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organized farm</strong></td>
<td>200</td>
<td>60</td>
<td>6.243**</td>
<td>80</td>
</tr>
<tr>
<td><strong>Private farm</strong></td>
<td>120</td>
<td>71</td>
<td>0.93**</td>
<td>200</td>
</tr>
</tbody>
</table>

Table 13. Incidence of organism between organized farms and Khatais

<table>
<thead>
<tr>
<th>Source of sample</th>
<th>Cows</th>
<th>Chi-square</th>
<th>Buffaloes</th>
<th>Chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organized farm</strong></td>
<td>200</td>
<td>60</td>
<td>9.453**</td>
<td>80</td>
</tr>
<tr>
<td><strong>Private farm</strong></td>
<td>120</td>
<td>71</td>
<td>0.93**</td>
<td>200</td>
</tr>
</tbody>
</table>

Table 12. Incidence of organism between organized and Private farms
### Table-14. Incidence of organism between Private farms and Khatauls

<table>
<thead>
<tr>
<th>Source of sample</th>
<th>Cows</th>
<th></th>
<th>Buffaloes</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of sample</td>
<td>No. of positive case</td>
<td>Chi-square</td>
<td>No. of sample</td>
<td>No. of positive case</td>
</tr>
<tr>
<td>Private farm</td>
<td>120</td>
<td>56</td>
<td>3.763&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>80</td>
<td>27</td>
</tr>
<tr>
<td>Khatal</td>
<td>120</td>
<td>71</td>
<td></td>
<td>120</td>
<td>49</td>
</tr>
</tbody>
</table>

### Table-15. Distribution of organism between animals

<table>
<thead>
<tr>
<th>Animals</th>
<th>No. of sample</th>
<th>No. of positive cases</th>
<th>percentage</th>
<th>Chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows</td>
<td>440</td>
<td>187</td>
<td>42.50</td>
<td>5.276**</td>
</tr>
<tr>
<td>Buffaloes</td>
<td>280</td>
<td>95</td>
<td>33.92</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>720</td>
<td>282</td>
<td>39.16</td>
<td></td>
</tr>
</tbody>
</table>

**P<0.01**
<table>
<thead>
<tr>
<th>TYPES</th>
<th>COWS</th>
<th>% DISTRIBUTION</th>
<th>CHI-SQUARE</th>
<th>BUFFALOES</th>
<th>% DISTRIBUTION</th>
<th>CHI-SQUARE</th>
<th>COWS</th>
<th>% DISTRIBUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRAND TOTAL</td>
<td>110</td>
<td></td>
<td></td>
<td>70</td>
<td></td>
<td></td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>

Table-16. Distribution of sub-clinical and clinical mastitis in cows and buffaloes.
<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of isolates</th>
<th>Coagulase test</th>
<th>Haemolytic</th>
<th>Pigmentation</th>
<th>Sugar fermentation test</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. haemolyticus</em></td>
<td>16</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>44</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>S. intermedis</em></td>
<td>82</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sugar fermentation test</th>
<th>Lactose</th>
<th>Glucose</th>
<th>Maltose</th>
<th>Sucrose</th>
<th>Mannitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactose</td>
<td>NR</td>
<td>VP</td>
<td>MR</td>
<td>Pigmentation</td>
<td>Haemolysis</td>
</tr>
</tbody>
</table>

Table 17. Biochemical characterization of coagulase positive Staphylococcus
<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of isolates</th>
<th>Coagulase test</th>
<th>Haemolytic</th>
<th>Pigmentation</th>
<th>MR</th>
<th>VP</th>
<th>MR</th>
<th>Gelatin liquefaction</th>
<th>NR</th>
<th>Proteolytic Enzyme</th>
<th>Sugar fermentation test</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. epidermidis</td>
<td>20</td>
<td>-</td>
<td>White</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S. simulane</td>
<td>8</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>S. saprophyticum</td>
<td>12</td>
<td>-</td>
<td>White</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 18. Biochemical characterizations of coagulase negative Staphylococcus
<table>
<thead>
<tr>
<th>Organisms</th>
<th>Kind of Organism</th>
<th>Strain</th>
<th>No of Strain</th>
<th>Treh</th>
<th>Salici</th>
<th>Mann</th>
<th>Sorbi</th>
<th>Sucr</th>
<th>Lacto</th>
<th>Gluc</th>
<th>Beta Haeomolytic</th>
<th>Narrow Zone</th>
<th>Narrow Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>agalactiae</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dysgalactiae</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uberis</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table-19 Biochemical Characterization of Streptococcus
<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of isolates</th>
<th>Beta haemolysis</th>
<th>Trehalose</th>
<th>Salicin</th>
<th>Raffinose</th>
<th>Sucrose</th>
<th>Mannitol</th>
<th>Sorbitol</th>
<th>Lactose</th>
<th>Glucose</th>
<th>Gelatin liquefaction</th>
<th>Growth in 6.5% Nacl broth</th>
<th>MR</th>
<th>VP</th>
<th>Catalase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corynebacterium ulcerans</td>
<td>16</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

Table-20 Biochemical characterizations of Corynebacterium
<table>
<thead>
<tr>
<th>Name of the organism</th>
<th>Adonitol</th>
<th>Arabinose</th>
<th>Rhamnose</th>
<th>Sucrose</th>
<th>Mannitol</th>
<th>Sorbitol</th>
<th>Lactose</th>
<th>Glucose</th>
<th>Gelatin liquefaction</th>
<th>Citrate</th>
<th>VP</th>
<th>MR</th>
<th>Indole</th>
<th>No of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteus vulgaris</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>90</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>12</td>
</tr>
<tr>
<td>E. coli</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>26</td>
</tr>
</tbody>
</table>

Table-21 Biochemical characterization of gram negative bacilli isolates
Table-22. Drug sensitivity pattern of different isolates

<table>
<thead>
<tr>
<th>Organism</th>
<th>No of isolates</th>
<th>Sensitivity pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GT</td>
</tr>
<tr>
<td>Coagulase positive staphylococcus</td>
<td>142</td>
<td>S</td>
</tr>
<tr>
<td>Coagulase negative staphylococcus</td>
<td>40</td>
<td>S</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Grams positive bacilli</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Grams negative bacilli</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Group A streptococcus</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Group B streptococcus</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Coagulase positive staphylococcus</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Coagulase negative staphylococcus</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Bacillus</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

GT - Gatifloxacin, SC - Sparfloxacin, CX - Cloxacillin, CT - Ceftriaxone, FO - Fosformicin, LO - Lomefloxacin, LA - Levofloxacin, GM - Gentamicin, AS - Ampicillin/Sulbactam, BA - Cotrimoxazole
GT-Gatifloxacin, SC-Sparfloxacin, CX-Cloxacillin, CT-Ceftriaxone, FO-Fosformicin, LO-Lomefloxacin, LA-Levofloxacin, GM-Gentamicin, AS-Ampicillin/Sulbactam, BA-Cotrimoxazole

Fig-1. Drug sensitivity pattern of coagulase positive Staphylococcus.
Drug used

GT - Gatifloxacin, SC - Sparfloxacin, CX - Cloxacillin, CT - Ceftriaxone, FO - Fosformicin, LA - Lomefloxacin, GM - Gentamicin, AS - Ampicillin/Sulbactam, BA - Cotrimoxazole

Fig-2. Drug sensitivity pattern of coagulase negative staphylococci

Sensitivity percentage (%)
Fig-3. Drug sensitivity pattern of streptococci

Drug used:

GT-Gatifloxacin, SC-Sparfloxacin, CX-Cloxacillin, CT-Ceftriaxone, FO-Fosformicin, LO-Lomefloxacin, LA-Levofloxacin, GM-Gentamicin, AS-Ampicillin/Sulbactam, BA-Cotrimoxazole

Sensitivity percentage (%)

Drug sensitivity pattern of streptococci

Fig-3.
Drug used

GT - Gatifloxacin, SC - Sparfloxacin, CX - Cloxacillin, CT - Ceftriaxone, FO - Fosformicin, LO - Lomefloxacin, LA - Levofloxacin, GM - Gentamicin, AS - Ampicillin/Sulbactam, BA - Cotrimoxazole

Sensitivity percentage (%)

Drugs

GT
SC
CX
CT
FO
LO
LA
GM
AS
BA

Fig. 4. Drug sensitivity pattern of gram positive bacilli
Fig-5. Drug sensitivity pattern of gram negative bacilli


Bar graph showing the drug sensitivity pattern of gram negative bacilli. The x-axis represents different drugs (GT, SC, CX, CT, FO, LA, GM, BA, AS) and the y-axis represents sensitivity percentage (%). The graph indicates the sensitivity levels of these drugs against gram negative bacilli.
Fig-3 showing culture on Mannitol salts agar

Fig-4 showing culture on Nutrient agar

Fig-5 showing culture on SDA

Fig-6 showing culture on Mac-conkey Agar

Fig-7 showing culture on EMB Agar
Fig-2 showing California Mastitis Test
Fig-1 showing Somatic cell count
Fig-8 showing Catalase test
Fig-9 showing Carbohydrate fermentation test
Fig-10 showing Oxidation-fermentation test
Fig-11 showing Indole, Methyl red, Voges proskauer and Citrate test
Fig-12 showing drug sensitivity test