

# Prevalence of Bovine mastitis bacteria and their antibiogram status

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Journal of Livestock Science (ISSN online 2277-6214) 17: 281-284

Received on 29/7/25; Accepted on 20/5/26; Published on 24/5/26

doi. 10.33259/JLivestSci.2026.281-284

## Abstract

Mastitis is a condition that affects large numbers of milch animals caused by several species of bacteria, fungi, and mycoplasma. A total number of 180 milk samples received from an organized farm in Jamdoli, Rajasthan state, India for a period of two years (2023-24) were subjected to California Mastitis Test (CMT), culture studies and for Gram's method of staining. The study revealed the Grams positive bacteria i.e. *Staphylococcus* Sp, *Streptococcus* Sp, *Corynebacterium* Sp and *Clostridium* Sp and the Grams negative bacteria i.e. *E. coli*, *Salmonella* Sp, *Yersinia* Sp, *Klebsiella* Sp and *Pseudomonas* Sp. The antibiogram study for these bacteria revealed sensitive patterns of antibiotics which were used regularly in bovine mastitis problem in this farm. The 16 antibiotics belongs to 7 antibiotic classes were subjected to antibiogram studies. In this study amikacin showed a sensitive value of 88.88%, followed by levofloxacin 77.77%, tetracycline 74%, ofloxacin 70.30%, ampicillin/ sulbactam 62.9%, gentamycin 62.9%, doxycycline 59.21%, imipenem 55.5%, co-trimoxazole (51.85%), amoxicillin/clavulanate (44.44), ceftriaxone (CTR)(33.33%), strepto-penicillin (29.6%), cefotaxime (CTX), 22.22%, cefixime (CFM) 14.81%, penicillin-G 11.11% and ampicillin 7.4%. The antibiogram studies with mastitis milk revealed the prevalence of multiple drug resistance (MDR) pattern for 7 antibiotic classes in this dairy herd.

**Keywords**-Bovine Mastitis; etiological agents; antibiogram; MDR status

## Introduction

Mastitis is the inflammation of the parenchyma of the mammary gland, characterized by physical, chemical and bacteriological changes in milk and pathological changes in glandular tissues. Jinu Manoj *et al* (2020) reported the highest prevalence of 54.49% in winter, followed by 69.61% by the rainy season in bovines with subclinical mastitis in Haryana state. Kumari *et al* (2018) reviewed subclinical mastitis of cattle with emphasis on prevalence, risk factors and economical losses in various states of India. Olufemi *et al* (2017) reported the reservoir status of the animal body for highly resistant bacteria. Mukana *et al* (2023) reported the prevalence of antimicrobial resistance as a health challenge to human beings by the year 2050. Manyi-Loh *et al* (2018) reported the antimicrobial resistance in human beings is due to continuous usage of antibiotics in food animal treatment. Since mastitis was reported frequently from these dairy animals an attempt was made to study the bacteria and their antibiogram status for better management of the dairy animals.

## Materials and methods

### Screening tests of milk samples and their antibiotic sensitivity studies

The studies were carried out in Apollo College of Veterinary Medicine, Jamdoli, Jaipur, Rajasthan State, India. The infected animals were treated as per the availability of the antibiotics in the market. The drug chloramphenicol not available, hence not used in the farm treatment practices. A total number of 180 milk samples were received within a period of 2 years from an organized dairy farm in Jaipur city, were initially screened by California mastitis test (CMT) for mastitis problem. All the 27 milk samples which were found positive with CMT were subjected to nutrient agar media culture studies followed by Gram's method of staining and organisms were classified for their gram positive and gram-negative characters as per their phenotypical characterization studies. The positive milk samples after CMT screening were subjected to antibiotic sensitivity test (ABST) on Muller Hinton agar Media by direct smearing method also and further implanted with antibiotic discs and read after 12 hours incubation at 37\*. The antibiotic sensitivity test was performed as per Kirby-Bauer antibiotic disc diffusion assay method on Mueller-Hinton agar plates using antibiotic discs of Hi-Media. The sensitivity and the resistance patterns of the organisms were recorded by comparing the diameter of growth inhibition zone as per the interpretation chart of the manufacturer (Hi-media lab Pvt Ltd) for the antibiotic discs. In these studies, 27 milk samples were subjected to antibiotic susceptibility studies using 16 antibiotic discs of 7 antibiotic classes. 1. Beta lactams (Ampicillin, Penicillin G, Amoxycillin, Strepto-penicillin, Amoxycylavate, Ampicillin/ Sulbactam). 2. Fluoroquinolones (Levofloxacin Ofloxacin) 3. Aminoglycosides (Amikacin, Gentamicin) 4. Sulpha (Co-Trimoxazole) 5. Tetracycline (Tetracycline, Doxycycline), 6. Cephalosporins (Ceftriaxone, Cefotaxime, Cefixime) 7. Carbapenem (Imipenem). The results were interpreted for their sensitivity as per the manufacturers' instructions.

## Results

Out of 180 milk samples, 27 samples found positive with CMT. The positive milk samples with CMT subjected to nutrient agar culture and Gram's method of staining for bacterial identification. The phenotypical characterization studies of the organisms revealed the prevalence of both Gram positive and Gram-negative bacteria. In the present study, the Grams positive bacteria i.e. *Staphylococcus* Sp, *Streptococcus* Sp, *Corynebacterium* Sp and *Clostridium* Sp and the Grams negative bacteria i.e. *E. coli*, *Salmonella* Sp, *Yersinia* Sp, *Klebsiella* Sp and *Pseudomonas* Sp were isolated. Out of 27 animals treated 22 did not recovered from mastitis due to MDR. The success rate of recovery was 20 percent in the affected animal population.

## Discussion

Mastitis is disease with serious economic implication in large and small ruminants (Ashish *et al.*, 2026). In this study, out of 180 milk samples received 27 samples, i.e. 15% were found positive for mastitis Bhanot *et al* (2012) reported a prevalence of 85.30% in cows and 78.1% in buffaloes respectively in Eastern Haryana. Charaya *et al* (2013) and Pankaj *et al* (2012) reported 33.7% and 64.21 % prevalence of subclinical mastitis in buffaloes and cows respectively from Hisar and Haryana. Jinu Manoj *et al* (2020) reported the highest prevalence of 54.49% in winter, followed by 69.61% by rainy season in Haryana bovines with subclinical mastitis. Mrinallee Devi and Dutta (2018) reported the prevalence of mastitis cases varies from place to place, herd to herd and country to country. Hossain *et al* (2017) suggested physiological, pathological, environmental or genetic factors as influencing factors to the occurrence of mastitis in dairy animals.

### Bacteria identified in bovine mastitis cases and their antibiogram status

In the present study, the Gram positive bacteria *i.e* *Staphylococcus* Sp, *Streptococcus* Sp, *Corynebacterium* Sp and *Clostridium* Sp and the Gram negative bacteria *i.e* *E. coli*, *Salmonella* Sp, *Yersinia* Sp, *Klebsiella* Sp and *Pseudomonas* Sp were isolated. In this study the order of sensitivity of the antibiotics which were used in this farm were : Amikacin (88.88%), Levofloxacin (77.77%), Tetracycline (74%), Ofloxacin (70.30%), Ampicillin/Sulbactam (62.9%), Gentamycin (62.9%), Doxycycline (59.21%), Imipenem (55.5%), Co-Trimoxazole (51.85%), Amoxicillin/clavulanate (44.44), Ceftriaxone (33.33%), Strepto-penicillin (29.6%), Cefotaxime (22.22%), Cefixime (14.81%), Penicillin-G (11.11%) and Ampicillin (7.4). Serdal Kurt and Funda Eski (2021) isolated Gram-negative *E. coli*, *Klebsiella*, *Enterobacter*, *Serratia*, *Pseudomonas*, and *Pasteurella* Sp, followed by Gram positive *Staphylococcus*, *Bacillus* Sp, *Streptococcus* Sp, *Actinomyces*, *Mycoplasma bovis* and fungi. The overall sensitivity showed chloramphenicol and streptomycin were the least effective antimicrobial agents and the most effective drugs were amikacin and kanamycin/cephalexin. The authors reported that the antibiogram on the bacterial isolates from infected milk samples showed different results, which may vary from agent to agent. The major microbes reported were *Staphylococcus* Sp, *Bacillus* Sp & *Pseudomonas* Sp. Rangini Manuel and Deepa (2022) reported the isolation of *S.aureus* (23.33%), *Streptococcus agalactiae* (10%), *E. coli* (6.6%) and *Enterobacter* (3.3%). The antibiogram showed 66.66% sensitivity to ceftriaxone/ tazobactam, 63.33% to cefoperazone, 56.66% to enrofloxacin, 53.33% to amoxicillin-clavulanate, 50% to tetracycline, 40% to gentamicin, 26.66% to ceftroxime, & 20% to co-trimazole and amoxicillin. Singh et al (2016) reported high resistance to the cephalosporins as per their antibiogram studies due to long term indiscriminate usage of this class. The susceptibility of Quinolones *i.e* levofloxacin and ofloxacin in this study were 77.77 and 70.30% respectively which agree with the reports of Singh et al (2016) who reported 71% resistance to ampicillin-sulbactam in buffaloes and 83% to amoxiclav in cattle population, whereas in this study the resistance to these antibiotics were less *i.e* 37.1% and 55.56% respectively. Studies on the susceptibility of the amikacin (70%) and gentamicin (67%) on the microbial mastitis pathogens were in close agreement with the observation of these studies. *i.e* 88.88% and 62.90% respectively. Mukana et al (2023) reported the reservoir status of the multiple drug-resistant bacteria in cattle and goats. The antibiogram associated with bovine mastitis varies from country to country as reported by authors as mentioned earlier. Hossain et al (2017) suggested that the therapeutic success of the mastitis is always low, because of the pathological changes occurring in the udder parenchyma which are related to mastitogenic bacterial related factors, indiscriminate antibiotics usage and poor husbandry farm practices. In this farm the mastitis cases were treated as per the availability of the antibiotics in the market in the absence of antibiogram studies. Hogeveen et al (2011) reported that the importance of the pathogens in mastitis cases varied between regions in the countries and farm to farm as per the husbandry practices followed. These studies also revealed varying antibiogram status and multiple drug resistance patterns for major antibiotics which were used regularly in bovine mastitis cases of this farm. The observation of these studies is in concurrence with the findings of the authors as discussed above.

### Conclusion

The study confirmed the prevalence of multiple drug resistance in bovine mastitis cases in the bovines for 7 antibiotic classes by antibiogram studies which needs further studies to confirm the antibiotic sensitivity status of all food animals to avoid zoonotic potential.

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