BOVINE MASTITIS: ROLE OF ANTIBIOTIC SENSITIVITY TESTING

Anshika Singh[1] & Nirbhay Bhawsar [2]
[1]B.V.Sc.-College of Veterinary Science & A.H. Rewa, NDVSU,[2]M.V.Sc.-Department of Extension Education, IVRI, Bareilly
*Corresponding author's e-mail: *drnirbhaybhawsar@gmail.com*
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ABSTRACT

India is the world's leading milk producer, and dairy farming is vital for rural livelihoods and food security (FAOSTAT, 2023). However, bovine mastitis remains one of the most widespread and economically important diseases affecting dairy cows and buffaloes (Halasa et al., 2007). Studies in India report that subclinical mastitis often affects over 40% of lactating animals, causing significant hidden production losses (Bangar et al., 2015). The disease reduces milk yield and quality, increases somatic cell counts, and raises treatment and culling costs (Seegers et al., 2003). In many cases, antibiotics are used empirically without laboratory confirmation, contributing to the rise of antimicrobial resistance (AMR) among major pathogens such as *Staphylococcus aureus* and *Escherichia coli* (Oliver et al., 2011; Marshall & Levy, 2011). AMR not only compromises treatment success but also poses risks to public health through the food chain (WHO, 2015). Antibiotic Sensitivity Testing (AST) provides a scientific approach to select effective drugs based on pathogen susceptibility, thereby improving cure rates and promoting responsible antimicrobial use (CLSI, 2020; Ruegg, 2017). Integrating routine AST into mastitis management is therefore essential for sustainable dairy production and protection of animal and public health in India.

KEYWORDS: IDF (International Dairy Federation), BAHS (Basic Animal Husbandry Statistics), antimicrobial resistance, Antibiotic Sensitivity Testing.

INTRODUCTION

According to the 20th Livestock Census (2019) published in the Basic Animal Husbandry Statistics, India has a total bovine population of about 303.8 million, consisting of approximately 193.5 million cattle and 109.85 million buffaloes (bovine species). India's cattle population accounts for a large share of the country's livestock and remains among the top in the world, with India ranking second globally for cattle numbers and first in buffalo population, where it holds the largest buffalo inventory in the world. India has made remarkable progress in the dairy sector and currently holds the position of the largest milk-producing country in the world. According to the Basic Animal Husbandry Statistics (BAHS) 2024–25, India's total milk production reached approximately 247.87 million tonnes in 2024–25, contributing nearly 25% of global milk production. Over the past decade (2014–15 to 2024–25), milk production has increased by more than 60%, reflecting consistent growth in the dairy industry. In addition, per capita milk availability has risen to about 485 grams per day in 2024–25, which is higher than the global average. These achievements highlight the crucial role of dairy farming in strengthening rural livelihoods, ensuring nutritional security, and supporting the national economy.

Despite this impressive growth, diseases such as mastitis continue to pose a major challenge to dairy productivity. Mastitis not only affects milk quality but also leads to significant reductions in milk yield, resulting in substantial economic losses. Research evidence from India clearly demonstrates its impact on production. A study published in the Indian Journal of Livestock Research (2020) reported that clinical mastitis reduced average daily milk yield by approximately 1.64 kg per day, with crossbred cows showing higher losses (2.37 kg/day) compared to indigenous cows (0.91 kg/day). Similarly, findings from the Indian Journal of Animal Sciences (ICAR) indicated that mastitis caused a 4.43% decline in total milk production in affected animals. Furthermore, field studies reported in Indian Farming (ICAR) revealed that subclinical mastitis alone can result in 10–25% milk production loss, often causing greater economic damage because it remains undetected for longer periods.

These findings underline that, although India leads the world in milk production, maintaining this position requires effective disease management. Adoption of scientific practices such as early diagnosis, proper milking hygiene, Antibiotic Sensitivity Testing (AST), and responsible antimicrobial use is essential to reduce mastitis-related losses, improve animal health, and ensure sustainable growth of the Indian dairy sector.

MASTITIS

Mastitis is a disease in which the udder (milk-producing gland) becomes swollen and inflamed, usually because germs enter through the teat canal. It can reduce milk production, change milk quality (clots, flakes, watery milk), and cause pain or fever in the animal. Sometimes it shows clear signs (clinical mastitis), and sometimes it remains hidden without visible symptoms (subclinical mastitis).

International Dairy Federation (IDF) defines mastitis as "An inflammation of the mammary gland, usually caused by bacterial infection, resulting in physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissue." (IDF, 2011)

In India, antibiotics are the primary treatment for bovine mastitis, with studies from organized dairy farms showing that β-lactams (penicillin and ampicillin-cloxacillin combinations) are most commonly used, followed by fluoroquinolones (enrofloxacin), aminoglycosides (gentamicin), and cephalosporins (ceftriaxone) according to ICAR field reports. These drugs are administered through intramammary infusion, systemic injections in severe cases, and as part of dry cow therapy to prevent new infections. However, frequent empirical use without culture and sensitivity testing has contributed to rising antimicrobial resistance among mastitis pathogens in Indian dairy herds, emphasizing the need for judicious antibiotic use.

Several research studies from India provide clear evidence of antimicrobial resistance (AMR) among mastitis-causing pathogens in dairy animals. A study conducted in Haryana on mastitic milk samples reported that Escherichia coli isolates showed resistance of up to 25% against commonly used antibiotics such as ampicillin, ciprofloxacin, and levofloxacin, and about 15% of isolates were identified as extended-spectrum β-lactamase (ESBL) producers, indicating resistance to multiple β-lactam drugs (Indian Journal of Dairy Science, ICAR). The same study found that nearly 32% of Staphylococcus aureus isolates were resistant to penicillin and ampicillin, and approximately 22% were identified as methicillin-resistant S. aureus (MRSA), reflecting reduced effectiveness of first-line antibiotics. Other Indian investigations have reported even higher resistance levels, with more than 70% of S. aureus isolates showing resistance to ampicillin, oxacillin, and tetracycline, and over 60% of E. coli isolates resistant to ampicillin and sulfamethoxazole-trimethoprim. Earlier studies from dairy farms also documented extremely high resistance to penicillin, in some cases reaching up to 90% or more among staphylococcal isolates. These findings collectively indicate the emergence of multidrug-resistant (MDR) mastitis pathogens in Indian dairy herds and highlight the urgent need for culture-based therapy, Antibiotic Sensitivity Testing (AST), and responsible antimicrobial stewardship to preserve drug efficacy.

ANTIBIOTIC SENSITIVITY TESTING (AST)

Antibiotic Sensitivity Testing (AST) is a laboratory test used to find out which antibiotic can effectively kill or stop the growth of bacteria causing an infection. In simple words, it helps doctors or veterinarians choose the right medicine instead of using antibiotics by guesswork.

The World Health Organization (WHO) describes antimicrobial susceptibility testing as:"Testing performed to determine whether a bacterial isolate is susceptible, intermediate, or resistant to specific antimicrobial agents." (WHO, Global AMR Surveillance System Manual)

Why AST is Important in Mastitis

Antibiotic Sensitivity Testing (AST) is a vital tool in the effective management of bovine mastitis, particularly in countries like India where antimicrobial resistance (AMR) is steadily rising. In many cases, mastitis treatment begins with broad-spectrum antibiotics on an empirical basis; however, different bacteria vary in their response to specific drugs. AST helps identify the exact causative pathogen and determines the most appropriate and effective antibiotic for treatment. By guiding veterinarians toward targeted therapy rather than trial-and-error approaches, AST improves cure rates and reduces the risk of treatment failure. It also plays a key role in limiting the emergence of resistant strains such as multidrug-resistant Staphylococcus aureus and Escherichia coli by preventing the unnecessary use of ineffective antimicrobials. Appropriate antibiotic selection further reduces repeated treatments and minimizes drug residues in milk, ensuring food safety and compliance with withdrawal periods. Moreover, AST supports antimicrobial stewardship initiatives by promoting rational and responsible drug use in dairy practice. Overall, the routine use of AST not only enhances animal health outcomes but also lowers economic losses associated with milk discard, veterinary expenses, and premature culling, thereby contributing to sustainable dairy production.

Different Types of Antibiotic Sensitivity Testing (AST) in Mastitis

1. Disk Diffusion Method (Kirby–Bauer Test)

This is the most widely used and economical method. Antibiotic-impregnated discs are placed on an agar plate inoculated with the bacterial isolate. After incubation, the zone of inhibition around each disc is measured. The organism is categorized as Sensitive, Intermediate, or Resistant based on standard guidelines (CLSI). It is commonly used in veterinary diagnostic labs.

2. Broth Dilution Method (MIC Test)

This method determines the Minimum Inhibitory Concentration (MIC) the lowest concentration of an antibiotic that inhibits visible bacterial growth. It can be performed as:

- Macro-broth dilution
- Micro-broth dilution (using microtiter plates)

MIC provides more precise quantitative results compared to disk diffusion.

3. E-test (Epsilometer Test)

This combines features of both disk diffusion and MIC testing. A plastic strip containing a gradient of antibiotic concentration is placed on an inoculated agar plate. The MIC value is read where the bacterial growth intersects the strip.

4. Automated AST Systems

Advanced laboratories use automated systems (e.g., VITEK-type systems) that provide rapid and standardized susceptibility results. These are more common in research or advanced diagnostic centers.

5. Molecular Methods (Genotypic AST)

Instead of measuring growth inhibition, molecular techniques (like PCR) detect resistance genes such as mecA (MRSA) or ESBL genes. These methods are faster but require specialized facilities.

MOST SIMPLE, FEASIBLE & ECONOMICAL AST METHOD FOR INDIAN DAIRY FARMERS

In the Indian dairy scenario, the most simple, feasible, and economical Antibiotic Sensitivity Testing (AST) method for mastitis is the Disk Diffusion (Kirby–Bauer) method. This technique is widely used in veterinary diagnostic laboratories because it is cost-effective, easy to perform, and does not require advanced equipment. It involves placing antibiotic-impregnated discs on an agar plate inoculated with bacteria isolated from mastitic milk, followed by measurement of the zone of inhibition to classify the organism as Sensitive, Intermediate, or Resistant according to standardized guidelines. In India, most state veterinary laboratories, veterinary colleges, and research institutes under the Indian Council of Agricultural Research (ICAR) commonly use this method for routine mastitis diagnosis due to limited resources and field applicability. The interpretation of results is generally done following standards provided by the Clinical and Laboratory Standards Institute (CLSI), which ensures reliability and comparability of results. Compared to MIC-based methods, disk diffusion is more affordable and practical for routine surveillance of antimicrobial resistance in bovine mastitis under Indian conditions.

CLINICAL SIGNS OF MASTITIS

Local (Udder) Signs- Mastitis commonly presents with noticeable local inflammatory changes in the affected udder quarter. The infected quarter becomes swollen, warm, and red due to increased blood flow and inflammation. On palpation, the animal shows pain and discomfort, and the affected teat or quarter may feel hard or firm compared to normal tissue. In some cases, the supramammary lymph nodes become enlarged as a response to infection. These signs collectively indicate active inflammation of the mammary gland and help in clinical diagnosis of mastitis.

Changes in Milk

In mastitis, noticeable changes occur in the milk's appearance and quality. The milk may contain clots, flakes, or even pus due to inflammation and infection of the mammary gland. It can become watery or abnormally thin instead of having its normal consistency. In more severe cases, the milk may appear blood-stained and may produce a foul odor. Along with these visible changes, there is usually a significant reduction in milk yield from the affected quarter or the entire animal.

Systemic Signs (Severe Cases)

In severe cases of mastitis, systemic signs may develop as the infection affects the entire body. The animal may exhibit fever along with a noticeable loss of appetite. Depression, dullness, and general weakness are commonly observed, and the animal may appear lethargic. Dehydration can occur due to reduced feed and water intake, especially in toxic cases. Additionally, there is often an increase in heart rate and respiratory rate as the body responds to infection and inflammation.

HOW TO PERFORM DISK DIFFUSION METHOD (KIRBY–BAUER TEST)

- The Disk Diffusion Method (Kirby–Bauer test) is a standard technique used to determine the antibiotic sensitivity of bacteria isolated from infections such as mastitis.
- Sample Collection & Bacterial Isolation-Milk is collected aseptically from the infected quarter before antibiotic treatment. The sample is cultured on suitable media to isolate the causative bacteria.
- Preparation of Bacterial Suspension-A pure bacterial colony is selected and suspended in sterile saline to match the 0.5 McFarland standard (standardized bacterial concentration).
- Inoculation of Agar Plate-A sterile swab is dipped into the suspension and evenly spread over the surface of Mueller–Hinton agar to create a uniform bacterial lawn.
- Placement of Antibiotic Discs-Using sterile forceps or a disc dispenser,

antibiotic-impregnated discs are placed on the agar surface at proper spacing.

- Incubation-The plate is incubated at 35–37°C for 18–24 hours.
- Measurement & Interpretation-After incubation, clear circular zones (zones of inhibition) appear around effective antibiotics. The diameter of these zones is measured in millimeters and interpreted as Sensitive (S), Intermediate (I), or Resistant (R) according to guidelines of the Clinical and Laboratory Standards Institute (CLSI).

BEST PRACTICES FOR EFFECTIVE MASTITIS CONTROL IN INDIAN DAIRY SCENARIO

In Indian dairy conditions, mastitis control mainly depends on simple and scientifically proven management practices. Maintaining proper milking hygiene, including washing and drying teats before milking and using post-milking teat dipping, significantly reduces new infections (National Dairy Development Board). Keeping sheds clean, providing dry bedding, and regularly removing dung help control environmental pathogens such as E. coli and Streptococcus species (Indian Council of Agricultural Research). Early detection through simple on-farm tests like the California Mastitis Test (CMT) allows identification of subclinical cases before they become severe (ICAR). Use of dry cow therapy at the end of lactation is proven to cure existing infections and prevent new infections during the dry period (NDDB). Adequate nutrition, particularly supplementation with selenium and vitamin E, supports udder immunity and lowers mastitis risk (ICAR).

These integrated practices are widely recommended in Indian dairy health management programs to improve milk quality and reduce economic losses due to mastitis.

CONCLUSION

India is the world's largest milk producer, and the dairy sector plays a crucial role in rural livelihoods, food security, and the national economy (FAOSTAT, 2023). Despite this growth, mastitis remains a major challenge, causing significant reductions in milk yield, deterioration in milk quality, increased somatic cell counts, and economic losses for farmers (Seegers et al., 2003). Subclinical mastitis, which often goes undetected, can reduce milk production by 10–25% and increase the risk of chronic infections and premature culling (Bangar et al., 2015).

The empirical use of antibiotics without laboratory confirmation has accelerated antimicrobial resistance (AMR) among key pathogens such as Staphylococcus aureus and Escherichia coli, reducing treatment efficacy and posing public health risks (Oliver et al., 2011; WHO, 2015). Antibiotic Sensitivity Testing (AST) enables targeted therapy by identifying effective drugs for specific pathogens, minimizing unnecessary antibiotic use and limiting the spread of resistant strains (CLSI, 2020; Ruegg, 2017).

Implementing integrated mastitis management strategies—including early detection, proper milking hygiene, clean housing, dry cow therapy, nutritional support, and routine AST—can substantially reduce production losses, improve milk quality, and enhance animal welfare (ICAR, 2022; NDDB, 2021). By adopting evidence-based and responsible practices, India can sustain its leadership in global milk production while promoting long-term dairy sector sustainability and safeguarding public health.

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