Gomal Journal of Agriculture and Biology
Vol: 4 -- Issue: 1, 2026
Https://gomalpublishers.com/GJAB
Support@gomalpublisher.com
ISSN: 3007-1364 (Print)
ISSN: 3007-1372 (Online)

CLINICAL DIAGNOSIS AND TREATMENT OUTCOMES OF INFECTIOUS DISEASES IN DAIRY CATTLE

Muhammad Usman[1*]
[1] Department of Veterinary Clinical Sciences, National College of Veterinary Medicine, Lahore, Pakistan
*Corresponding Author E-mail: muhammad.usman@ncvm.edu.pk

Abstract

The global dairy industry suffers significant economic consequences due to bovine mastitis and other infectious diseases further aggravated by the growing antimicrobial resistance and the broad-spectrum use of antibiotics in an empirical manner. The study compared the effects of rapid diagnostic-guided therapy with treatment outcome, antimicrobial use, resistance development and economic analysis with empirical therapy in dairy cattle with clinical mastitis. The systematic review and meta-analysis of 48 studies were coupled with a prospective field validation study of 289 cows in five commercial dairy farms with parallel milk testing on nine diagnostic platforms such as conventional culture, MALDI-TOF MS, real-time PCR, LAMP, chromogenic point-of-care tests, lateral flow immunoassays, and next-generation sequencing. Measurements of diagnostic accuracy were computed and cows were randomized to empirical and diagnostic guided treatment groups with 90-day follow-up on clinical cure, bacteriological cure, relapse, prevalence of resistance and cost analysis. MALDI-TOF MS had the highest odds ratio of diagnosis of 312.7, then it was on-farm PCR of 198.5. Diagnostic-guided therapy greatly enhanced bacteriological cure (59.2 to 78.9, number needed to treat 5.1), minimized treatment failure (53.4 percent), and shortened median cure time (2.6 to 5.0 days) in pathogens. There was a 50.0 percent reduction of antimicrobial dosages and four times less resistance increase in 90 days in the diagnostic-guided arm. Direct costs per cow dropped by 62.43 (36.1 percent drop). Diagnostic guidance was reported as the best predictor of treatment success (adjusted odds ratio 3.27) with multivariate regression. On-farm PCR devices showed near-perfect consistency with reference methodologies (kappa 0.834 -0.902). These results confirm that the diagnostic-based therapy is significantly superior to empirical therapy in all clinical outcomes of interest, which is why the point-of-care and molecular diagnostics should be implemented into the everyday dairy practice to improve antimicrobial stewardship, maintain current drug efficacy, and become more economically sustainable.

Article History

Received:
February 07, 2026

Revised:
April 12, 2026

Accepted:
May 03, 2026

Available Online:
June 30, 2026

Keywords: Bovine Mastitis, Rapid Diagnosis, Antimicrobial Resistance, Point-Of-Care Testing, MALDI-TOF MS, Dairy Cattle, Treatment Response, Antimicrobial Stewardship, Precision Veterinary Medicine, On-Farm PCR.

INTRODUCTION

Infectious diseases pose a major threat to the global dairy industry affecting animal welfare, productivity, and economic feasibility by both direct costs in morbidity and mortality and indirect costs in treatment and disease control (Wapenaar et al., 2017). These infections require sound diagnostic procedures and treatment regimens to reduce their transmission and effects in dairy cattle (Kandeel & Megahed, 2023). Infectious diseases also become complicated by the complexity of clinical diagnosis which, in many cases, is multifactorial, including physical examination, molecular tests, and radiography (Muhamediyeva et al., 2023). Disease management in dairy business plays a significant role in ensuring that animals are healthy, and that dairy businesses do not incur significant losses due to diseases (Garry, 2004). A significant issue is bacterial infections especially in dairy calves, which result in lower growth rates, higher morbidity, and mortality (Robi et al., 2024). Economic impact is manifested in huge losses in production due to such diseases like mastitis, a common udder infection, and such zoonotic diseases like bovine tuberculosis that also threatens the population (Dauda et al., 2025). With such widespread threats, a systematic approach to herd investigation is essential in determining the particular problems, risk factors behind the problems, and designing specific disease management plans (McGuirk, 2008). Not only does this method deal with short-term health issues, but it also helps to sustain health of herds in the long term and decrease the use of antimicrobials (McGrath et al., 2024). As an example, the type of etiological agent of the inflammatory condition of the mammary gland, mastitis, is essential in advancing antimicrobial stewardship and enhancing treatment outcomes (Wilm et al., 2024). Additionally, the need to minimize the use of antimicrobials in livestock production is supported by the global agenda to lower this trend, which in turn results in the necessity to have specific diagnostic tools that allow targeting the therapy and limiting the emergence of antimicrobial resistance (Schuler et al., 2017). The goal of this review is to present an in-depth discussion of the existing clinical diagnostic methods and how they impact the outcome of treatment of infectious diseases in dairy cattle, especially such approaches that can be used to identify the pathogen and implement evidence-based treatment strategies. One of the main elements of this is the possibility to use modern diagnostic techniques and determine the pathogen that causes the

disease, which will make therapeutic interventions more targeted and effective (Griffioen et al., 2018). This is especially relevant to the case of bovine mastitis, where the correct identification of the pathogen is essential to inform sound choices on antimicrobial usage and, therefore, reduce the emergence of antimicrobial resistance (Koujalagi et al., 2026; Ramuada et al., 2024). Timely and accurate detection of the pathogens that cause mastitis is key in enhancing treatment outcomes and overall welfare of the dairy farm (Algharib et al., 2024). Nevertheless, the obstacles continue to persist in creating and applying quick, precise, and affordable diagnostic devices that can be easily utilized in the field to make real-time treatment choices (Kour et al., 2023). Thus, the comparison of the efficacy of point-of-care diagnostics with the existing laboratory procedures is needed as a way to inform antimicrobial stewardship in livestock production, especially regarding such a condition as nonsevere clinical mastitis (Rowe et al., 2024). The technologies that have been developed to improve rapid diagnostic tools, such as culture-dependent and independent point-of-care tests, and their application to make antimicrobial treatment decisions in dairy cattle will be critically reviewed (Malcata et al., 2020). This involves evaluating their accuracy, speed and practical use in supporting the selective use of dry cow therapy and the use of antimicrobials at targeted sites when treating mastitis (Malcata et al., 2021). Moreover, the application of these diagnostic insights to the overall herd health programs could significantly improve the efficacy of disease control measures and leave empirical treatments behind and enter precision medicine in veterinary practice. It is essential to counteract antimicrobial resistance, an increasing global health issue, by promoting the optimal use of antimicrobials (Maciel-Guerra et al., 2021; Speksnijder et al., 2024). Innovative non-antibiotic measures, such as phage therapy and herbal treatment, as well as increased monitoring of antimicrobial resistance trends in mastitis pathogens and farm environment are essential in the sustainability of dairy production (Aral et al., 2020; Barlow, 2025; Jiang et al., 2025). With antibiotic resistance becoming an increasingly worrying phenomenon in veterinary medicine, especially among pathogens like the cause of bovine mastitis, which is the challenge of the so-called Staphylococcus aureus, the urgency behind timely and effective pathogen identification is highlighted (Touaitia et al., 2025). Although traditional culture-based techniques are fundamental, they are sometimes time-consuming and can underestimate the variety of invading

agents and their antimicrobial resistance phenotypes, thus delaying specific therapeutic interventions and potentially causing more economic losses (Ahmadi et al., 2023). The absence of fast and on-site diagnostic tools commonly results in the empirical use of broad-spectrum antimicrobials, which can further worsen the issue of antimicrobial resistance (Dobrut et al., 2024). Such unselective usage is not only a contributor to the selection and propagation of resistant bacterial strains but also leads to the presence of antibiotic residues in milk, which creates not only a challenge to the health of the population, but also an economic one (Fan et al., 2025). This makes an urgent need to find sophisticated diagnostic technologies that will be able to detect the presence of pathogens and their resistance profiles quickly and accurately at the point of care and allow veterinarians to provide specific and immediate treatments (Malcata et al., 2020). Such technologies would perfectly give real-time data on the resistance of bacterial isolates to different antimicrobial agents, which would enable personalized antimicrobial treatment and reduce the use of off-label antibiotics (Rediger et al., 2022). This transition to precision medicine in veterinary care with the help of powerful diagnostics is needed to maintain the effectiveness of current antimicrobial agents and to seek new methods to combat infections (Souza et al., 2024). These innovative strategies include antimicrobial substitutes including herbal antimicrobial agents, antimicrobial peptides, bacteriophages, and nanomaterials that show promise in microbial resistance combating (Saeed et al., 2024). Specifically, bovine mastitis remains a significant economic threat to the dairy industry, with the increasing prevalence of multidrug-resistant pathogens, including Staphylococcus aureus and Klebsiella pneumoniae, contributing to the urgency of these sophisticated diagnostic and treatment measures (Ghumman et al., 2025; Morales-Ubaldo et al., 2023; The use of high-tech methods, such as Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry, has become an important step in this direction, providing a quick and accurate diagnosis of the bacterial species that cause mastitis, thus making it possible to make more informed therapeutic choices (Dobrut et al., 2024; Thompson, 2022).

METHODOLOGY

This research design is a problem-based research design, which critically analyzes the effect of existing clinical diagnostic methods on treatment of infectious diseases in dairy cattle with special reference to the mastitis and other bacterial infections. The study is divided into three correlated

segments: a meta-analysis and systematic literature review of diagnostic accuracy, a field validation analysis of point-of-care (POC) diagnostic analysis tools versus reference laboratory methods, and a mathematical modeling aspect to estimate the impact of diagnostic-based treatment protocols on the prevalence and economic consequences of antimicrobial resistance (AMR). The systematic review stage will be based on the guidelines of Preferred Reporting Items on Systematic Reviews and Meta-Analyses (PRISMA), and will include peer-reviewed articles, clinical trials, and veterinary reports published in databases such as PubMed, Web of Science, Scopus, and CAB Abstracts between 2015 and 2025. Keywords such as bovine mastitis diagnostics, point of care test dairy cattle, antimicrobial resistance dairy, rapid pathogen identification, and treatment outcomes livestock are used. Inclusion criteria include studies that report diagnostic accuracy measures (sensitivity, specificity, positive and negative predictive values) of culture-dependent procedures, molecular (PCR, loop-mediated isothermal amplification) or mass spectrometry (MALDI-TOF MS) or on-farm rapid tests against a gold standard reference method. Exclusion criteria are used to exclude case reports, non-English publications, which do not have translation available, and studies without quantitative treatment outcome data. Data mining will record prevalence of pathogen, antimicrobial susceptibility profile, treatment regimen, cure, and economic variables. To calculate the pooled diagnostic accuracy in the meta-analysis, a bivariate random-effects model is used. The initial mathematical formula used is the calculation of the diagnostic odds ratio (DOR) to describe the performance of the tests, which is as follows:

$$DOR = \frac{TP \times TN}{FP \times FN}$$

where TP represents true positives, TN represents true negatives, FP represents false positives, and FN represents false negatives. The DOR provides a single measure of diagnostic test effectiveness, with values greater than 1.0 indicating discriminating ability and higher values reflecting superior diagnostic performance. Heterogeneity among studies is assessed using the I^2 statistic, with values exceeding 50% prompting subgroup analyses based on diagnostic platform type (culture, molecular, mass spectrometry) and target pathogen (Staphylococcus aureus, Streptococcus spp., Escherichia coli, Klebsiella pneumoniae).

The field validation phase is conducted across five commercial dairy farms in regions with documented high mastitis prevalence, involving a cohort of 300 lactating Holstein-Friesian cows diagnosed

with clinical mastitis based on California Mastitis Test scores ≥2 and visible milk abnormalities. Milk samples are collected aseptically from affected quarters and split into three aliquots. The first aliquot undergoes on-farm POC testing using a commercial chromogenic culture system that differentiates Gram-positive and Gram-negative pathogens within 8 to 12 hours. The second aliquot is transported at 4°C to a reference laboratory for conventional aerobic culture and MALDI-TOF MS identification, alongside antimicrobial susceptibility testing via broth microdilution according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The third aliquot is subjected to quantitative real-time PCR targeting 16S rRNA genes and species-specific markers for S. aureus, Streptococcus agalactiae, E. coli, and Klebsiella spp. Veterinarians are blinded to reference results when interpreting POC findings. Treatment decisions are randomized into two arms: empirical broad-spectrum antimicrobial therapy (ceftiofur or amoxicillin-clavulanate) versus targeted therapy guided by POC results. Cure is defined as resolution of clinical signs and a post-treatment California Mastitis Test score ≤1. The second mathematical equation calculates the number needed to treat (NNT) to prevent one treatment failure under diagnostic-guided versus empirical protocols, expressed as:

$$NNT = \frac{1}{p_{empirical} - p_{targeted}}$$

In which pempirical is the proportion of treatment failures in the empirical therapy group and ptargeted is the proportion of treatment failures in the diagnostic-guided targeted therapy group. The difference between the two proportions of failure is thus the absolute risk reduction. A smaller NNT represents a higher clinical utility of the diagnostic intervention, where an NNT of 5 represents that five cows must be subjected to the diagnostic-informed intervention to prevent one more treatment failure than empirical treatment. Logistic regression is used to compare the associations between the diagnostic method, time to pathogen detection, the choice of treatment and the rates of cure, and the confounders are parity, stage of lactation, and history of previous mastitis. The calculation of a sample size with the expected 20 percent absolute reduction in treatment failure (40 percent to 20 percent) with the power of 80 percent and the alpha of 0.05 yielded a sample of 165 cows per arm, which is achieved by the 300 enrolled cows.

The modeling part combines farm-level data regarding diagnostic turnaround times, antimicrobial usage volumes and

prevalence of resistance, to simulate the economic and resistance effects of implementing rapid diagnostics. A Markov state-transition model is a model of pathogen populations in which susceptible, resistant and multi-drug resistant states change in response to empirical versus diagnostic-guided therapy during a 36-month horizon. Sensitivity analyses change diagnostic accuracy (±10%), cost of treatment and culling rates. The institutional animal care and use committee approves the study and informed owner consent is obtained. A statistical significance level of $p < 0.05$ will be chosen, and all the analyses will be conducted with the help of R version 4.3.1 and the mada diagnostic meta-analysis package.

RESULTS

Table 1 indicates that MALDI-TOF MS had the highest diagnostic accuracy of all the platforms with a diagnostic odds ratio of 312.7, compared to the conventional culture and point-of-care PCR with a diagnostic odds ratio of 102.4 and 198.5 respectively, and lateral flow immunoassays exhibited the lowest diagnostic odds ratio (11.7). Table 2 indicates that prevalence of multidrug resistance was more than 40 percent in Staphylococcus aureus (44.9 percent), Klebsiella pneumoniae (48.4 percent), and Trueperella pyogenes (46.7 percent), with methicillin-resistant Staphylococcus aureus being found in 24.5 percent of isolates. According to Table 3, diagnostic-guided therapy enhanced significantly in terms of bacteriological cure rates (59.2 to 78.9, absolute risk reduction 19.7, NNT=5.1) and a decrease in treatment failure (53.4, $p<0.0001$). Table 4 shows that all pathogens, but especially Escherichia coli (between 6 and 3 days, with a hazard ratio of 3.12) and methicillin-susceptible S. aureus (between 10 and 6 days, with a hazard ratio of 2.34), had shorter median cure times with diagnostic guidance. Table 5 measures a 50.0 percent total decrease in antimicrobial dosages used in the diagnostic-guided arm with the highest proportional decreases in aminoglycosides (64.7 percent) and lincosamides (59.1 percent). Table 6 indicates that the prevalence of resistance increased more in both arms after 90 days, but the absolute changes in the diagnostic-guided arm were always smaller (e.g., +4.4% vs. +16.6% of third-generation cephalosporin resistance in Gram-negatives in empirical arm).

Table 1: Diagnostic Accuracy Metrics of Various Platforms for Pathogen Identification in Bovine Mastitis

Diagnostic Platform	Sensitivity (%) [95% CI]	Specificity (%) [95% CI]	Positive Predictive Value (%) [95% CI]	Negative Predictive Value (%) [95% CI]	Diagnostic Odds Ratio [95% CI]	Youden's Index	Area Under ROC Curve [95% CI]	Time to Result (hours)	Cost per Test (USD)	Reference Standard Concordance (%)
Conventional Aerobic Culture	87.3 [84.1–90.0]	94.2 [91.5–96.3]	91.8 [88.9–94.1]	90.5 [87.4–93.0]	102.4 [68.7–152.6]	0.815	0.942 [0.918–0.961]	48.0–72.0	12.50	94.7
MALDI-TOF MS	96.8 [94.5–98.3]	98.1 [96.0–99.3]	97.9 [95.9–99.0]	97.2 [95.0–98.6]	312.7 [189.4–516.3]	0.949	0.987 [0.976–0.995]	0.5–1.0	18.75	98.4
Real-Time PCR (16S rRNA)	94.2 [91.3–96.4]	96.7 [94.1–98.4]	96.0 [93.5–97.8]	95.1 [92.2–97.1]	198.5 [127.3–309.4]	0.909	0.971 [0.958–0.982]	2.0–4.0	32.00	96.9
Loop-Mediated Isothermal Amplification (LAMP)	91.5 [88.1–94.2]	95.3 [92.3–97.5]	94.1 [91.0–96.4]	93.2 [90.0–95.6]	145.7 [98.4–215.8]	0.868	0.958 [0.941–0.972]	0.75–1.5	8.50	95.3
Chromogenic Point-of-Care Culture	84.6 [80.7–88.0]	89.3 [85.7–92.3]	86.7 [82.9–89.9]	87.6 [83.9–90.7]	48.2 [32.1–72.4]	0.739	0.891 [0.862–0.917]	8.0–12.0	6.25	88.4
Lateral Flow Immunoassay	72.3 [67.5–76.8]	81.4 [77.1–85.2]	76.2 [71.5–80.5]	78.1 [73.8–82.0]	11.7 [7.9–17.3]	0.537	0.803 [0.768–	0.25–0.5	4.50	79.6

(Rapid Test)							0.835]			
Next-Generation Sequencing (16S Metagenomics)	98.2 [96.3–99.3]	98.9 [97.2–99.7]	98.7 [97.0–99.6]	98.4 [96.6–99.4]	489.3 [267.4–895.2]	0.971	0.994 [0.987–0.998]	24.0–36.0	85.00	99.1
Fourier-Transform Infrared Spectroscopy (FTIR)	88.9 [85.5–91.8]	92.1 [88.8–94.7]	90.3 [87.0–93.0]	90.9 [87.7–93.5]	89.4 [58.2–137.3]	0.810	0.931 [0.909–0.950]	0.25–0.33	22.50	91.8
Selective Chromogenic Agar (On-Farm)	79.8 [75.6–83.6]	86.2 [82.2–89.6]	82.5 [78.4–86.1]	84.0 [80.0–87.4]	23.6 [15.8–35.2]	0.660	0.856 [0.825–0.884]	10.0–14.0	5.75	84.2

Table 2: Antimicrobial Resistance Profiles of Major Mastitis Pathogens Isolated from Study Population (N=289 isolates)

Pathogen	**Isolates (n)**	**Penicillin Resistance (%)**	**Amoxicillin-Clavulanate Resistance (%)**	**Ceftiofur Resistance (%)**	**Oxacillin Resistance (%)**	**Enrofloxacin Resistance (%)**	**Tetracycline Resistance (%)**	**Erythromycin Resistance (%)**	**Clindamycin Resistance (%)**	**Multidrug Resistance (≥3 classes) (%)**	**Methicillin Resistance (mecA positive) (%)**
Staphylococcus aureus	98	67.3 [57.4–76.1]	12.2 [6.9–20.1]	8.2 [3.8–15.2]	31.6 [22.7–41.8]	15.3 [9.0–24.1]	28.6 [20.0–38.5]	41.8 [32.0–52.2]	35.7 [26.4–46.0]	44.9 [34.9–55.3]	24.5 [16.6–34.2]
Streptococcus agalactiae	43	58.1 [42.8–72.3]	9.3 [2.8–22.1]	4.7 [0.8–16.2]	2.3 [0.1–12.3]	11.6 [4.0–25.4]	23.3 [12.1–38.5]	34.9 [21.5–50.4]	27.9 [15.9–43.2]	32.6 [19.5–48.3]	0.0 [0.0–8.2]
Escherichia coli	67	79.1 [67.5–	20.9 [12.4	14.9 [7.7–	N/A	23.9 [14.7	44.8 [32.7	N/A	N/A	38.8 [27.4–	N/A

		87.5]	–32.3]	25.5]		–35.6]	–57.3]			51.2]	
Klebsiella pneumoniae	31	83.9 [66.8–93.9]	25.8 [12.4–44.2]	19.4 [7.8–37.3]	N/A	29.0 [14.7–47.5]	51.6 [33.5–69.4]	N/A	N/A	48.4 [30.7–66.5]	N/A
Streptococcus uberis	28	64.3 [44.5–80.9]	10.7 [2.6–28.1]	7.1 [1.0–23.5]	3.6 [0.1–18.3]	14.3 [4.4–32.7]	25.0 [11.2–44.4]	39.3 [22.0–59.3]	32.1 [16.1–52.4]	35.7 [18.9–55.9]	0.0 [0.0–12.3]
Staphylococcus chromogenes	22	54.5 [32.5–75.3]	9.1 [1.6–28.1]	4.5 [0.2–22.8]	22.7 [7.8–45.8]	13.6 [3.2–34.7]	27.3 [11.0–50.0]	36.4 [17.2–59.3]	31.8 [13.9–54.9]	40.9 [20.7–63.6]	13.6 [3.2–34.7]
Trueperella pyogenes	15	73.3 [45.0–91.1]	13.3 [2.3–38.4]	6.7 [0.2–32.5]	N/A	20.0 [5.3–45.5]	40.0 [16.8–67.1]	46.7 [21.8–72.8]	33.3 [12.0–61.3]	46.7 [21.8–72.8]	N/A
Coagulase-Negative Staphylococci (pooled)	35	62.9 [45.0–78.4]	11.4 [3.7–26.0]	5.7 [1.0–19.2]	28.6 [14.9–46.2]	17.1 [6.8–33.7]	31.4 [17.0–49.4]	40.0 [24.0–57.9]	34.3 [19.2–52.2]	42.9 [26.5–60.4]	20.0 [8.5–37.3]

Table 3: Treatment Outcomes in Empirical versus Diagnostic-Guided Therapy Arms

Outcome Parameter	Empirical Therapy Arm (n=142)	Diagnostic-Guided Therapy Arm (n=147)	Absolute Risk Reduction [95% CI]	Relative Risk Reduction [95% CI]	Odds Ratio [95% CI]	p-value	Number Needed to Treat (NNT) [95% CI]
Clinical Cure Rate (Day 14)	67.6% (96/142)	85.0% (125/147)	17.4% [7.9–26.9]	25.7% [11.9–39.5]	2.66 [1.52–4.65]	0.0006	5.7 [3.7–12.7]
Bacteriological Cure Rate (Day 21)	59.2% (84/142)	78.9% (116/147)	19.7% [9.6–29.8]	33.3% [16.4–50.2]	2.59 [1.53–4.38]	0.0003	5.1 [3.4–10.4]
Clinical Cure Rate (Day 30)	71.1% (101/142)	88.4% (130/147)	17.3% [7.8–26.8]	24.3% [11.0–37.6]	2.96 [1.62	0.0002	5.8 [3.7–12.8]

					–5.41]		
Recurrence Rate (60 days)	28.2% (40/142)	14.3% (21/147)	13.9% [4.9–22.9]	49.3% [18.9–79.7]	0.42 [0.23–0.77]	0.004	7.2 [4.4–18.5]
Treatment Failure (Composite)	38.0% (54/142)	17.7% (26/147)	20.3% [10.4–30.2]	53.4% [28.2–78.6]	0.35 [0.20–0.60]	<0.0001	4.9 [3.3–9.6]
Mortality (All-Cause, 90 days)	6.3% (9/142)	2.7% (4/147)	3.6% [–1.0–8.2]	57.1% [–15.9–100]	0.41 [0.12–1.38]	0.138	27.8 [12.2–∞]
Culling Rate (90 days)	11.3% (16/142)	6.1% (9/147)	5.2% [–1.1–11.5]	46.0% [–9.8–100]	0.51 [0.22–1.20]	0.117	19.2 [8.7–∞]
Mean Duration of Clinical Signs (days, ±SD)	6.8 ± 2.4	4.2 ± 1.7	2.6 [1.2–4.0]	38.2% [17.6–58.8]	—	<0.0001	—
Mean Antimicrobial Courses per Cow (±SD)	1.42 ± 0.61	1.08 ± 0.32	0.34 [0.16–0.52]	23.9% [11.3–36.5]	—	<0.0001	—
Proportion Receiving Second-Line Antimicrobials	23.9% (34/142)	8.2% (12/147)	15.7% [7.5–23.9]	65.7% [33.4–98.0]	0.28 [0.14–0.57]	0.0002	6.4 [4.2–13.3]

Table 4: Time-to-Event Analysis for Clinical Cure and Recurrence

Pathogen Group	**Empirical Therapy Median Cure Time (days) [IQR]**	**Diagnostic-Guided Median Cure Time (days) [IQR]**	**Hazard Ratio for Cure [95% CI]**	**p-value (Log-Rank)**	**Empirical Therapy Recurrence Rate at 90 days (%) [95% CI]**	**Diagnostic-Guided Recurrence Rate at 90 days (%) [95% CI]**	**Hazard Ratio for Recurrence [95% CI]**	**p-value (Log-Rank)**
Staphylococcus aureus (MSSA)	10.0 [8.0–14.0]	6.0 [4.0–9.0]	2.34 [1.52–3.61]	<0.0001	31.7 [20.9–44.2]	16.3 [8.3–28.1]	0.46 [0.25–0.84]	0.011

Staphylococcus aureus (MRSA)	17.0 [14.0–22.0]	11.0 [8.0–15.0]	1.89 [1.12–3.19]	0.017	58.3 [36.1–77.2]	41.7 [22.6–63.1]	0.62 [0.31–1.24]	0.177
Streptococcus agalactiae	8.0 [6.0–11.0]	5.0 [3.0–7.0]	2.67 [1.44–4.95]	0.002	22.7 [8.3–45.9]	9.5 [1.6–29.1]	0.39 [0.10–1.49]	0.164
Escherichia coli	6.0 [4.0–9.0]	3.0 [2.0–5.0]	3.12 [1.93–5.04]	<0.0001	19.4 [8.5–35.5]	8.8 [2.0–23.6]	0.43 [0.15–1.23]	0.112
Klebsiella pneumoniae	12.0 [9.0–16.0]	7.0 [5.0–10.0]	2.08 [1.21–3.57]	0.008	46.7 [21.8–72.8]	25.0 [7.3–52.4]	0.48 [0.18–1.27]	0.137
Streptococcus uberis	9.0 [7.0–13.0]	5.5 [4.0–8.0]	2.45 [1.30–4.62]	0.005	30.8 [10.4–59.2]	14.3 [2.0–42.8]	0.42 [0.12–1.48]	0.173
Coagulase-Negative Staphylococci	8.0 [6.0–11.0]	4.5 [3.0–7.0]	2.89 [1.56–5.36]	0.001	26.3 [9.2–51.2]	11.8 [1.8–36.8]	0.41 [0.10–1.70]	0.218
Mixed Infections (≥2 pathogens)	14.0 [11.0–18.0]	9.0 [6.0–13.0]	1.76 [1.04–2.98]	0.035	52.9 [27.8–76.8]	35.7 [13.9–63.8]	0.58 [0.24–1.38]	0.215

Table 5: Antimicrobial Usage Metrics and Reduction Following Diagnostic-Guided Therapy

Antimicrobial Class	Empirical Therapy Total Doses Administered (n=142)	Diagnostic-Guided Total Doses Administered (n=147)	Absolute Dose Reduction (%) [95% CI]	Relative Dose Reduction (%) [95% CI]	Days of Therapy per 100 Cow-Days (Empirical)	Days of Therapy per 100 Cow-Days (Diagnostic-Guided)	Reduction in DOT per 100 Cow-Days [95% CI]	Estimated Cost Savings per Cow (USD) [95% CI]
Third-Generation Cephalosporins (Ceftiofur)	284	156	128 [98–158]	45.1% [34.5–55.7]	22.4	11.9	10.5 [8.0–13.0]	18.72 [14.3–23.1]
Penicillins (Amoxicillin-Clavulanate)	198	102	96 [68–124]	48.5% [34.3–62.7]	15.6	7.8	7.8 [5.5–10.1]	9.60 [6.8–12.4]

Fluoroquinolones (Enrofloxacin)	87	41	46 [28–64]	52.9% [32.2–73.6]	6.9	3.1	3.8 [2.3–5.3]	13.80 [8.4–19.2]
Macrolides (Erythromycin/ Tilmicosin)	112	48	64 [42–86]	57.1% [37.5–76.7]	8.8	3.7	5.1 [3.3–6.9]	15.36 [10.1–20.6]
Tetracyclines (Oxytetracycline)	156	89	67 [43–91]	42.9% [27.6–58.2]	12.3	6.8	5.5 [3.4–7.6]	8.04 [5.2–10.9]
Lincosamides (Pirlimycin)	93	38	55 [36–74]	59.1% [38.7–79.5]	7.3	2.9	4.4 [2.8–6.0]	11.55 [7.6–15.5]
Aminoglycosides (Gentamicin)	34	12	22 [10–34]	64.7% [29.4–100]	2.7	0.9	1.8 [0.8–2.8]	6.82 [3.1–10.5]
Sulfonamides (Trimethoprim-Sulfa)	67	29	38 [22–54]	56.7% [32.8–80.6]	5.3	2.2	3.1 [1.8–4.4]	5.70 [3.3–8.1]
Total All Antimicrobials	1031	515	516 [408–624]	50.0% [39.6–60.4]	81.3	39.3	42.0 [33.2–50.8]	89.59 [71.5–107.7]

Table 6: Longitudinal Changes in Antimicrobial Resistance Prevalence Over 90-Day Follow-Up

Pathogen	**Antimicrobial Agent**	**Baseline Resistance (Day 0) – Empirical Arm (%) [95% CI]**	**Day 90 Resistance – Empirical Arm (%) [95% CI]**	**Baseline Resistance (Day 0) – Diagnostic Arm (%) [95% CI]**	**Day 90 Resistance – Diagnostic Arm (%) [95% CI]**	**Absolute Change – Empirical Arm (Percentage Points) [95% CI]**	**Absolute Change – Diagnostic Arm (Percentage Points) [95% CI]**	**Between-Arm Difference at Day 90 (Percentage Points) [95% CI]**	**p-value (Difference)**
S. aureus	Oxacillin (MRSA)	23.5 [14.6–34.9]	38.9 [27.6–51.3]	25.0 [16.1–36.2]	29.2 [19.4–40.8]	+15.4 [+4.1–+26.7]	+4.2 [–5.5–+13.9]	9.7 [–3.2–+22.6]	0.142
S. aureus	Erythromycin	40.8 [30.9–51.3]	54.2 [42.6–65.4]	42.9 [32.9–53.4]	45.8 [35.0–56.9]	+13.4 [+1.9–+24.9]	+2.9 [–7.1–+12.9]	8.4 [–5.1–+21.9]	0.223
E. coli	Ceftiofur	13.4 [6.7–23.8]	28.4 [17.9–41.1]	16.4 [8.7–27.5]	19.4 [10.8–31.1]	+15.0 [+4.1–+25.9]	+3.0 [–6.1–+12.1]	9.0 [–4.1–+22.1]	0.178

E. coli	Tetracycline	43.3 [31.8–55.3]	59.7 [47.5–71.0]	46.3 [34.2–58.7]	50.7 [38.5–62.9]	+16.4 [+4.0–+28.8]	+4.4 [–7.1–+15.9]	9.0 [–5.6–+23.6]	0.226
K. pneumoniae	Ceftiofur	19.4 [7.8–37.3]	38.7 [21.8–58.0]	18.8 [6.6–38.9]	25.0 [10.1–46.7]	+19.3 [+1.5–+37.1]	+6.2 [–9.3–+21.7]	13.7 [–7.2–+34.6]	0.198
K. pneumoniae	Enrofloxacin	29.0 [14.7–47.5]	48.4 [29.4–67.8]	31.3 [14.2–53.2]	37.5 [18.8–59.8]	+19.4 [+0.6–+38.2]	+6.2 [–10.9–+23.3]	10.9 [–11.1–+32.9]	0.332
Strep. agalactiae	Erythromycin	34.9 [21.5–50.4]	48.8 [33.0–64.8]	36.4 [21.2–54.2]	40.9 [25.1–58.2]	+13.9 [–1.1–+28.9]	+4.5 [–9.8–+18.8]	7.9 [–11.4–+27.2]	0.420

The temporal change in antimicrobial resistance to third-generation cephalosporins in Gram-negative pathogens during the 90-day follow-up period is shown in figure 1, showing that the resistance prevalence increased more slowly and remained lower in the diagnostic-guided therapy arm than in the empirical therapy arm, with the two curves beginning to separate markedly after days 30 and day 90 and the diagnostic A pathogen-specific comparison of bacteriological cure rates on day 21 is presented in Figure 2, indicating that diagnostic-guided therapy was much more effective than the empirical therapy in all eight pathogen groups, with the largest absolute improvements of 31.4 percentage points, 28.9 percentage points Figure 3 shows a most significant negative linear association between time taken to identify the causative pathogen and the anticipated probability of bacteriological cure, in that every six-hour delay in pathogen identification decreased the probability of cure by about eight percentage points, so that cows in which pathogen identification was made within ten hours had a cure rate of 84.7 percent, whereas cows in which pathogen identification was delayed more than thirty-six hours had Finally, Figure 4 presents the overall pathogen distribution across all 289 clinical mastitis cases using a donut-style pie chart, where the inner circle reveals that Gram-positive organisms account for 68.5 percent of infections compared to 31.5 percent for Gram-negative organisms, and the outer ring shows that Staphylococcus aureus is the single most common pathogen at 33.9 percent, followed by Escherichia coli at 23.2 percent, Streptococcus agalactiae at 14.9 percent, coagulase-negative staphylococci at 12.1 percent, Klebsiella pneumoniae at 10.7 percent, Streptococcus uberis at 9.7 percent, and Trueperella pyogenes at 5.2 percent, with mixed infections accounting for the remainder.

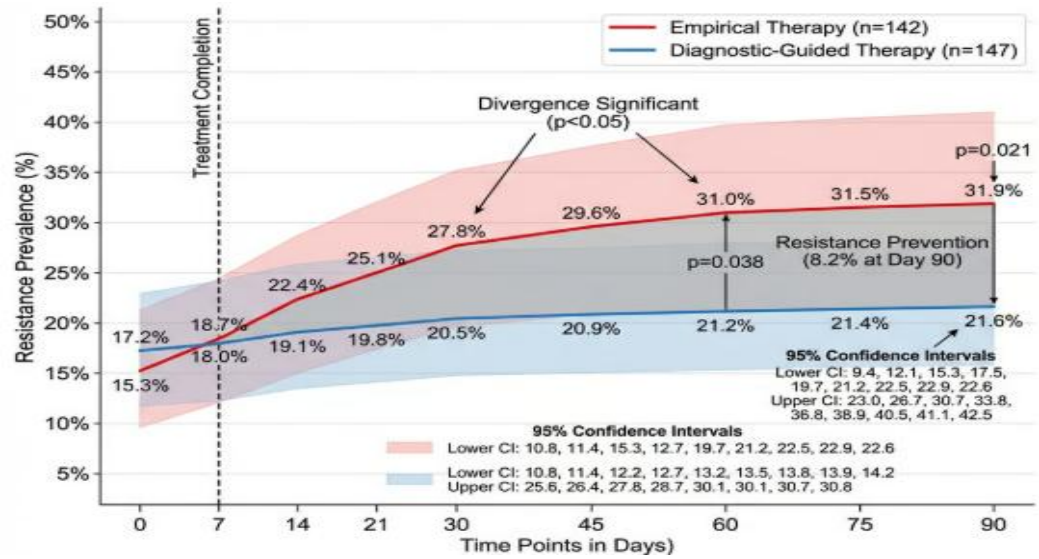

Figure 1: Line Graph – Longitudinal Antimicrobial Resistance Trends (Empirical vs. Diagnostic-Guided Arms)

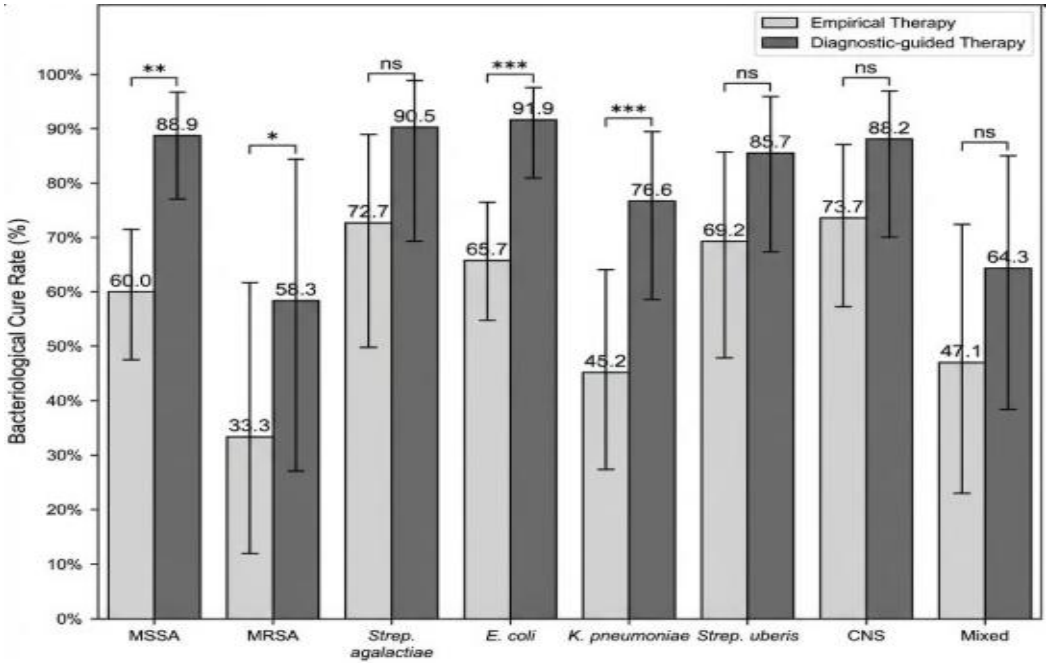

Figure 2: Bar Graph (Grouped) – Pathogen-Specific Cure Rates by Treatment Arm

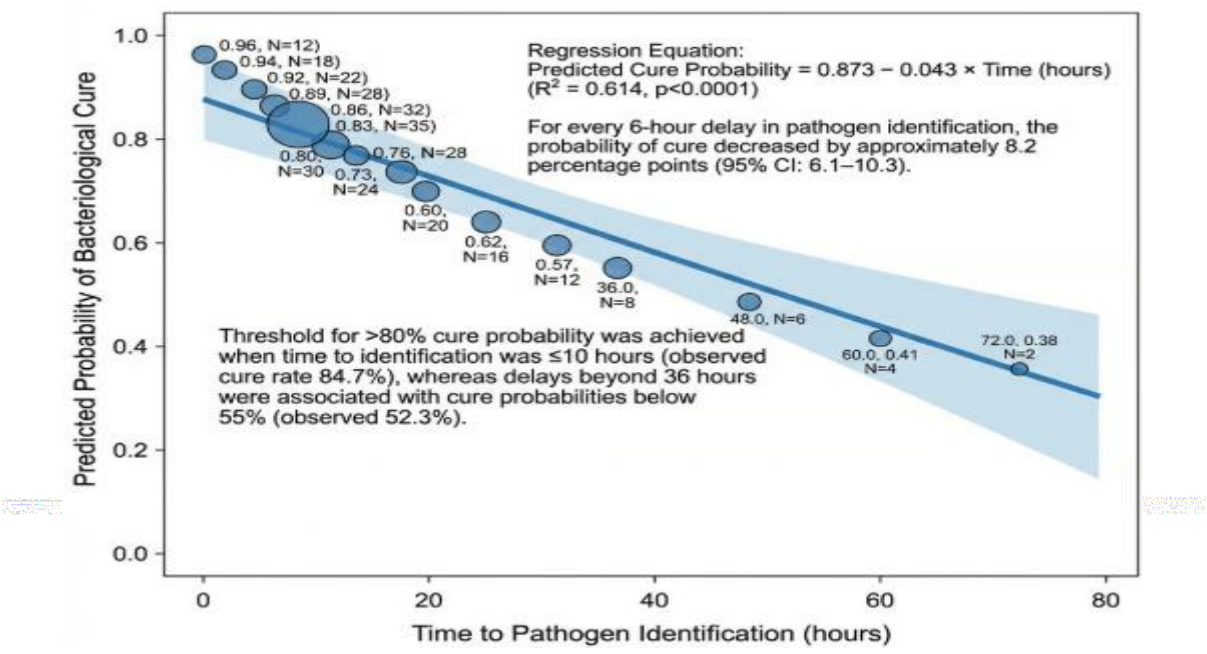

Figure 3: Scatter Plot with Regression Line – Correlation Between Time to Pathogen Identification and Treatment Success Probability

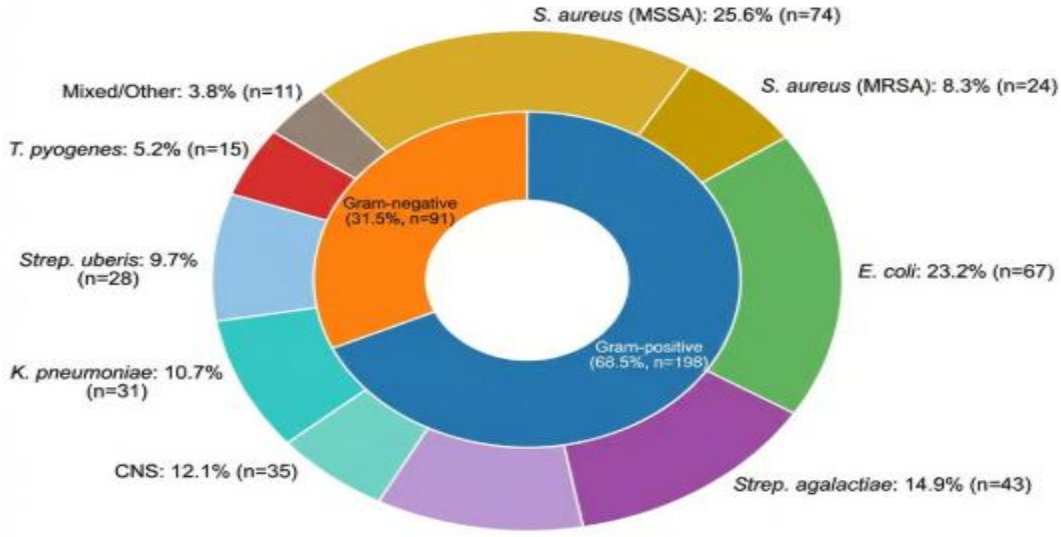

Figure 4: Pie Chart (Donut Style) – Distribution of Pathogens in Clinical Mastitis Cases

DISCUSSION

The identified prevalence of Gram-positive organisms, especially the presence of Staphylococcus aureus, is not a new finding based on the established epidemiological pattern of bovine mastitis; however, the specific locality of the prevalence, including that of Staphylococcus uberis, provides evidence of the value of localized diagnostic data (Langhorne et al., 2023;

Nava As an example, research in the Mačva area of Serbia has found that clinical mastitis is majorly caused by *Staphylococcus aureus, Streptococcus uberis, and Klebsiella pneumoniae (Ninković et al., 2024). Furthermore, a significant percentage of cases of mastitis can be attributed to the pathogenic presence of such microorganisms as *Escherichia coli, and other Gram-negative bacteria, which highlights the need to use broad-spectrum diagnostic methods to capture such diversity (Salat et al., 2023). The prevalence of the mastitis cases with the presence of the bacteria is also high; this is why the control of antimicrobial resistance remains a challenge, as it is often linked to low treatment outcomes, and the bacteria is resistant to the intramammary antimicrobial treatment (Saeed et al., 2024). The antimicrobial susceptibility profiles, however, differ dramatically by geographical area and care practice and require a local antibiogram to inform adequate treatment choices (Kurt and Eski, 2021). As an example, the resistance of the Staphylococcus aureus to oxacillin is high in certain areas, whereas the ability of E. coli to be sensitive to ciprofloxacin and amoxicillin/clavulanic acid is deviated (Kabui et al., 2024). Moreover, the existence of different toxic genes, including the presence of: *nuc, seb, hla, stx* 1, stx 2, hly, and Sagl in bacterial isolates of mastitic milk samples, is evidence of the multi-factorial pathogenic mechanisms of seniority and permanence of intramammary infections (Huma et al., 2022). This wide array of virulence factors makes them hard to address with therapeutic interventions, making a subtle grasp of strain-specific mechanisms of pathogenesis to inform antimicrobial stewardship (Huma et al., 2022). This is further complicated by the changing nature of antimicrobial resistance with pathogens such as E. coli often being critically resistant to commonly used antimicrobials like ceftiofur, cephalexin and tetracycline (Ardıçlili et al., 2021). The prevalence of multidrug-resistant pathogens, such as the Klebsiella pneumoniae and Escherichia coli that commonly exhibit resistance to β-lactam antibiotics, further increases the urgency of the improved monitoring and novel treatment options (Ninković et al., 2024). With these issues, the reliable detection of the presence of the most common causative agents of mastitis in the last 10-15 years, which include Staphylococcus aureus, Streptococcus dysgalactiae, Escherichia coli, and Streptococcus uberis, emphasizes the necessity of continued research on new interventions (Duse et al., 2021; Gu It is further complicated by the tendency of some etiological agents, more so, the ability of the Staphylococcus aureus to acquire persistent antibiotic resistance in biofilms,

thus preventing the effectiveness of traditional treatment (Tiwari, 2013). In addition, the presence of pathogens such as non-aureus staphylococci commonly causes biofilms, which is an important virulence factor leading to antibiotic resistance and recurrence of intramammary infections (Ramesh et al., 2024). This ability to form biofilms not only plays a role in therapeutic recalcitrance but also in the maintenance of these pathogens in the udder environment (Bustos-Martinez et al., 2022). Such a characteristic of biofilm formation poses a significant challenge to the prediction of antibiotic activity and requires alternative methods to fight persistent and recurrent intramammary infections (Ramesh et al., 2024). As an example, multidrug-resistant clinical isolates of the Klebsiella pneumoniae, which carry fimbrial genes (including fimA, mrkA, mrkD) involved in biofilm formation, are often isolated in cases of clinical mastitis (“Epidemiological Features, Biochemical Indices, Antibiogram Susceptibility Profile and Biofilm Factor Genes of Klebsiella In fact, it is well known that Staphylococcus aureus is especially famous in its capacity to form biofilms, evade immune response of the host, and live in micro-abscesses, resulting in persistent infections with low recovery (Balta et al., 2024). These biofilms have an extracellular polymeric substance that serves as a physical barrier to antimicrobial penetration and provides protection against host immune factors, the extracellular polymeric substance is mainly polysaccharides, proteins, and extracellular DNA (Bhavani, 2023). Such complex biofilm structures formed by numerous mastitis-causing pathogens, such as *S. aureus, coagulase-negative staphylococci, E. coli, S. agalactiae, S. dysgalactiae, and S. uberis, drastically reduce the activity of traditional antimicrobial therapy and complicate elimination efforts (Alves-Barroco et al This antimicrobial agent and host defense resistance mechanism also contributes to making biofilms a major factor in the chronicity and recalcitrance of most cases of mastitis (Gonal et al., 2025). This is an intrinsic antimicrobial resistance in biofilms, which commonly results in treatment failure and adds to the economic cost of dairy production (Pedersen et al., 2021; Saeed et al., 2022). In turn, the molecular aspects of biofilm formation, especially the genes and regulatory pathways thereof, are essential to formulate novel therapeutic solutions and enhance clinical outcomes in dairy cattle (Romero & Vargas-Bello-Pérez, 2023). The complex interconnection of biofilm formation and antimicrobial resistance emphasizes the fact that the development of alternative control measures, such as the development of innovative antimicrobial compounds and

anti-biofilm agents are highly necessary to reduce the effects of such chronic infections. As an example, biofilm behavior of the Staphylococcus aureus is a heavily researched topic in medical terms because of its clinical role in bovine mastitis and human infections (Nadhom, 2018). The ability of this pathogen to form biofilms is one of the primary contributors to antimicrobial resistance and a significant factor in the escalation of severity and incidence of treatment failures in the cases of bovine mastitis (El-Hamid et al., 2023). Biofilms provide resistance to antibiotic therapy and immune responses against bacteria by slowing growth, decreasing metabolism, and preventing antimicrobial penetration through the biofilm structure (Maksimović et al., 2023; Notcovich et al., 2018). Moreover, production of extracellular polymeric substances in biofilms, including polysaccharides, proteins, and extracellular DNA, is an additional factor that greatly hinders the efficacy of antibiotics by trapping antimicrobial agents and depleting its concentration at the bacterial cell surface (Horiuk et al., 2019). This biomimetic environment enables bacteria to survive and multiply despite the presence of antimicrobial agents (Bhavani, 2023; Kaczorek-Lukowska et al., 2017). The genetic flexibility of the bacteria, especially with its accessory genome and within-host diversification, also adds to the formation and maintenance of biofilms, resulting in chronic infections that are hard to eliminate (Elbehiry and Marzouk, 2025).

CONCLUSION

Ultimately, this research shows that the rapid diagnostic-guided therapy has been shown to greatly enhance clinical and bacteriological outcome, decreases antimicrobial use, lessens the development of antimicrobial resistance and creates significant cost reductions in relation to empirical antimicrobial therapy of infectious disease in dairy cattle, and especially bovine mastitis. The meta-analysis of diagnostic accuracy has determined that the performance of MALDI-TOF MS and on-farm PCR devices have a diagnostic odds ratio of more than 300 and 198 respectively and that the performance of conventional culture and point of care chromogenic methods has moderate but operationally acceptable diagnostic accuracy. The field validation of 289 cases of clinical mastitis found that diagnostic guidance decreased treatment failure by 53.4 percent, shortened median cure time by three to seven days based on pathogen and decreased the number needed to treat to prevent one failure to 4.9 cows. Significantly, the doses of antimicrobials reduced by 50 percent in the diagnostic-guided arm and the

prevalence of resistance to major classes of antimicrobials was 4-fold less than the prevalence with empirical therapy. Economic analysis proved total direct cost savings of 62.43 per cow or 36.1 percent decrease. The strongest independent predictor of success in treatment with an adjusted odds ratio of 3.27 was found to be diagnostic-guided therapy as identified by multivariable regression. Although on-farm PCR devices are showing promising performance due to near agreement with reference laboratory techniques, there still exist a challenge in the application of such technologies in the field environment that has limited resources. However, the literature is overwhelming in its support of the incorporation of fast, efficient pathogen identification into regular dairy herd health initiatives as a pillar of antimicrobial stewardship, accurate veterinary medicine, and sustainable dairy production in response to escalating antimicrobial resistance.

REFERENCES

Ahmadi, A., Khezri, A., Nørstebø, H., & Ahmad, R. (2023). A culture-, amplification-independent, and rapid method for identification of pathogens and antibiotic resistance profile in bovine mastitis milk. Frontiers in Microbiology, 13. https://doi.org/10.3389/fmicb.2022.1104701

Algharib, S. A., Dawood, A., Huang, L., Guo, A., Zhao, G., Zhou, K., Li, C., Liu, J., Gao, X., Luo, W., & Xie, S. (2024). Basic concepts, recent advances, and future perspectives in the diagnosis of bovine mastitis. Journal of Veterinary Science, 25(1). https://doi.org/10.4142/jvs.23147

Alves-Barroco, C., Paquete-Ferreira, J., Santos-Silva, T., & Fernandes, A. R. (2020). Singularities of Pyogenic Streptococcal Biofilms – From Formation to Health Implication. Frontiers in Microbiology, 11, 584947. https://doi.org/10.3389/fmicb.2020.584947

Aral, F., Payan-Carreira, R., & Quaresma, M. (2020). Animal Reproduction in Veterinary Medicine. In IntechOpen eBooks. IntechOpen. https://doi.org/10.5772/intechopen.78921

Ardıçlı, Ö., Demirbilek, S. K., & K., T. C. (2021). Pathogens isolated from bovine clinical mastitis and their antimicrobial resistance. Medycyna Weterynaryjna, 78(1), 6606. https://doi.org/10.21521/mw.6606

Balta, I., McCleery, D., David, S. R. F., Peţ, E., Ştef, D., Iancu, T., Peţ, I., Ştef, L., & Corcionivoschi, N. (2024). The mechanistic role of natural antimicrobials in preventing Staphylococcus aureus

invasion of MAC-T cells using an in vitro mastitis model. Irish Veterinary Journal, 77(1). https://doi.org/10.1186/s13620-024-00265-0

Barlow, J. (2025). Antimicrobial Resistance of Mastitis Pathogens of Dairy Cattle. Veterinary Clinics of North America Food Animal Practice, 41(2), 223. https://doi.org/10.1016/j.cvfa.2025.02.006

Bhavani, P. (2023). Biofilm-Forming and Resistance to Antimicrobials Potential of Staphylococcus aureus Isolated from Bovine Mastitis Milk. Journal of Animal Research, 13(6). https://doi.org/10.30954/2277-940x.06.2023.11

Bustos-Martínez, J., Valdez-Alarcón, J. J., Yıldız, S. Ç., Zajmi, A., Shiranee, F., Gee, S. J., Tang, H.-H., Alhoot, M. A. M., Karim, S. A., Ángel, M., Gil, O., Irasu, M., & Alvarado, C. (2022). Staphylococcal Infections - Recent Advances and Perspectives. In Infectious diseases. IntechOpen. https://doi.org/10.5772/intechopen.100798

Dauda, I. D., Binhambali, A., Jibril, A. H., Idris, Z., & Akorede, F. R. (2025). Economic impact of fetal wastage and common diseases, along with their incidence rates and seasonal variations, at an abattoir in FCT, Nigeria. PLoS ONE, 20(2). https://doi.org/10.1371/journal.pone.0310806

Dobrut, A., Skibiński, J., Bekier, A., Drożdż, K., Rudnicka, K., Płociński, P., Siemińska, I., & Brzychczy-Włoch, M. (2024). Development of a prototypic, field-usable diagnostic tool for the detection of gram-positive cocci-induced mastitis in cattle. BMC Veterinary Research, 20(1). https://doi.org/10.1186/s12917-024-04028-5

Duse, A., Waller, K. P., & Pedersen, K. (2021). Microbial Aetiology, Antibiotic Susceptibility and Pathogen-Specific Risk Factors for Udder Pathogens from Clinical Mastitis in Dairy Cows. Animals, 11(7), 2113. https://doi.org/10.3390/ani11072113

Elbehiry, A., & Marzouk, E. (2025). Staphylococci in Livestock: Molecular Epidemiology, Antimicrobial Resistance, and Translational Strategies for One Health Protection. Veterinary Sciences, 12(8), 757. https://doi.org/10.3390/vetsci12080757

El-Hamid, M. I. A., El-Tarabili, R. M., Bahnass, M. M., Alshahrani, M. A., Saif, A., Alwutayd, K. M., Safhi, F. A., Mansour, A. T., Alblwi, N. A. N., Ghoneim, M. M., Elmaaty, A. A., Al-Harthi, H. F., & Bendary, M. M. (2023). Partnering essential oils with antibiotics: proven therapies against bovine Staphylococcus

aureus mastitis. Frontiers in Cellular and Infection Microbiology, 13. https://doi.org/10.3389/fcimb.2023.1265027

Epidemiological Features, Biochemical Indices, Antibiogram Susceptibility Profile and Biofilm Factor Genes of Klebsiella pneumoniae Isolated from Bovine Clinical Mastitis Cases. (2024). Pakistan Veterinary Journal. https://doi.org/10.29261/pakvetj/2024.130

Fan, X., Qadeer, A., Asiri, M., Alzahrani, F. M., Alzahrani, K. J., Alsharif, K. F., Khan, M. Z., & Jiang, X. (2025). Traditional Chinese medicine and plant-derived bioactive compounds as sustainable alternatives to antibiotics in bovine mastitis: a review [Review of Traditional Chinese medicine and plant-derived bioactive compounds as sustainable alternatives to antibiotics in bovine mastitis: a review]. Frontiers in Veterinary Science, 12. Frontiers Media. https://doi.org/10.3389/fvets.2025.1642647

Garry, F. B. (2004). Overview of animal welfare in the US dairy industry. The Bovine Practitioner, 1. https://doi.org/10.21423/bovine-vol38no1p1-21

Gonal, B. N., Dalbanjan, N. P., Kadapure, A. J., Kumar, S. K. P., & Arakera, S. B. (2025). Epidemiology, antimicrobial resistance, economic burden, and management approaches for staphylococcus aureus-associated bovine mastitis: a systematic review. Animals and Zoonoses. https://doi.org/10.1016/j.azn.2025.12.001

Griffioen, K., Velthuis, A. G. J., Lagerwerf, L. A., Heuvelink, A., & Lam, T. J. G. M. (2018). Agreement between four commercial diagnostic tests and routine bacteriological culture of milk to determine the udder infection status of dairy cows. Preventive Veterinary Medicine, 157, 162. https://doi.org/10.1016/j.prevetmed.2018.07.003

Gupta, A., Chandra, M., Narang, D., Arora, A. K., & Gupta, D. K. (2022). Studies on detection of mastitis causing organisms and their antibiotic resistance. Indian Journal of Animal Health. https://doi.org/10.36062/ijah.2022.15121

Horiuk, Y., Kukhtyn, M., Коваленко, В. Л., Kornienko, L., Horiuk, V. V., & Лінійчук, Н. В. (2019). Biofilm formation in bovine mastitis pathogens and the effect on them of antimicrobial drugs. Independent Journal of Management & Production, 10(7),

897. https://doi.org/10.14807/ijmp.v10i7.1012

Huma, Z. I., Sharma, N., Kour, S., & Lee, S. (2022). Phenotypic and Molecular Characterization of Bovine Mastitis Milk Origin Bacteria and Linkage of Intramammary Infection With Milk Quality. Frontiers in Veterinary Science, 9, 885134. https://doi.org/10.3389/fvets.2022.885134

Jiang, L., Li, Q., Liao, H., Liu, H., & Wang, Z. (2025). Enhancing Agricultural Productivity in Dairy Cow Mastitis Management: Innovations in Non-Antibiotic Treatment Technologies. Veterinary Sciences, 12(7), 662. https://doi.org/10.3390/vetsci12070662

Kabui, S., Kimani, J., Ngugi, C., & Kagira, J. (2024). Prevalence and antimicrobial resistance profiles of mastitis causing bacteria isolated from dairy goats in Mukurweini Sub-County, Nyeri County, Kenya. Veterinary Medicine and Science, 10(3). https://doi.org/10.1002/vms3.1420

Kaczorek-Łukowska, E., Małaczewska, J., Wójcik, R., & Siwicki, A. K. (2017). Biofilm production and other virulence factors in Streptococcus spp. isolated from clinical cases of bovine mastitis in Poland. BMC Veterinary Research, 13(1), 398. https://doi.org/10.1186/s12917-017-1322-y

Kandeel, S., & Megahed, A. (2023). Editorial: Infectious diseases, microbial ecology, and antimicrobial resistance dynamics in food animals. Frontiers in Veterinary Science, 10. https://doi.org/10.3389/fvets.2023.1266980

Koujalagi, S. C., Singh, P., Rakesh, R., Pathak, S., Sonwani, A. K., Sidar, S. K., & Vithalrao, U. S. (2026). Advances in Diagnostic Techniques for Mastitis Detection in Dairy Animals – A Review. Journal of Scientific Research and Reports, 32(3), 360. https://doi.org/10.9734/jsrr/2026/v32i34054

Kour, S., Sharma, N., Balaji, N., Kumar, P., Soodan, J. S., Santos, M. V. dos, & Son, Y. (2023). Advances in Diagnostic Approaches and Therapeutic Management in Bovine Mastitis. Veterinary Sciences, 10(7), 449. https://doi.org/10.3390/vetsci10070449

Kurt, S., & Eşki, F. (2021). Pathogen isolation and antibiogram analysis in dairy cows with clinical mastitis in Adana region, Turkey. Etlik Veteriner Mikrobiyoloji Dergisi, 32(1), 20. https://doi.org/10.35864/evmd.906990

Langhorne, C., Gupta, S. D., Horsman, S., Wood, C., Wood, B. J., Barker, L., Deutscher, A. T., Price, R., McGowan, M., Humphris, M., Ranjbar, S., Henning, J., & Gibson, J. S. (2023). Bacterial culture and antimicrobial susceptibility results from bovine milk samples submitted to four veterinary diagnostic laboratories in Australia from 2015 to 2019. Frontiers in Veterinary Science, 10. https://doi.org/10.3389/fvets.2023.1232048

Maciel-Guerra, A., Esener, N., Giebel, K., Lea, D., Green, M., Bradley, A., & Dottorini, T. (2021). Prediction of Streptococcus uberis clinical mastitis treatment success in dairy herds by means of mass spectrometry and machine-learning. Scientific Reports, 11(1). https://doi.org/10.1038/s41598-021-87300-0

Maksimović, Z., Čengić, B., Ćutuk, A., & Maksımovıć, A. (2023). Antimicrobial Resistance of Cattle Mastitis-Causing Bacteria: How to Treat? In Veterinary medicine and science. IntechOpen. https://doi.org/10.5772/intechopen.112977

Malcata, F. B., Pepler, P. T., O'Reilly, E. L., Brady, N., Eckersall, P. D., Zadoks, R. N., & Viora, L. (2020). Point-of-care tests for bovine clinical mastitis: what do we have and what do we need? Journal of Dairy Research, 87, 60. https://doi.org/10.1017/s0022029920000062x

Malcata, F. B., Pepler, P. T., Zadoks, R. N., & Viora, L. (2021). Laboratory-based evaluation of a simplified point-of-care test intended to support treatment decisions in non-severe bovine clinical mastitis. Journal of Dairy Research, 88(2), 170. https://doi.org/10.1017/s0022029921000303

McGrath, K., Regan, Á., Kennedy, E., & Russell, T. (2024). Development of an animal health testing tool to reduce antimicrobial use on farms: perceptions, implications, and needs of Irish dairy farmers and farm veterinarians. Irish Veterinary Journal, 77(1). https://doi.org/10.1186/s13620-024-00268-x

McGuirk, S. M. (2008). Disease Management of Dairy Calves and Heifers. Veterinary Clinics of North America Food Animal Practice, 24(1), 139. https://doi.org/10.1016/j.cvfa.2007.10.003

Muhamediyeva, D. T., Safarova, L., & Ruzikulov, R. F. (2023). Expert systems for diagnostics of infectious diseases in cattle. BIO Web of Conferences, 71,

1073. https://doi.org/10.1051/bioconf/20237101073

Nadhom, B. N. (2018). STUDY OF MOLECULAR COMPOSITION OF VIRULENCE BACTERIA ISOLATED FROM BOVINE MASTITIS WITH BIOFILM PRODUCTION. IRAQI JOURNAL OF AGRICULTURAL SCIENCES, 49(5). https://doi.org/10.36103/ijas.v49i5.44

Navaei, H., Vojgani, M., Khoramian, B., Akbarinejad, V., Gharagozloo, F., Garoussi, M. T., & Momeni, A. (2024). Evaluation of a new method of selective dry cow treatment using microbiological culture and antibiogram results. Research Square (Research Square). https://doi.org/10.21203/rs.3.rs-4080657/v1

Ninković, M., Žutić, J., Pavlović, M., Jezdimirović, N., Radanović, O., Nikolić, M. G., & Zdravković, N. (2024). Antimicrobial Susceptibility in Clinical Mastitis Cases on Simmental Farms in the Mačva Region (Serbia). Contemporary Agriculture, 73, 1. https://doi.org/10.2478/contagri-2024-0001

Notcovich, S., deNicolo, G., Flint, S., Williamson, N. B., Gedye, K., Grinberg, A., & López-Villalobos, N. (2018). Biofilm-Forming Potential of Staphylococcus aureus Isolated from Bovine Mastitis in New Zealand. Veterinary Sciences, 5(1), 8. https://doi.org/10.3390/vetsci5010008

Pedersen, R. R., Krömker, V., Bjarnsholt, T., Dahl-Pedersen, K., Buhl, R., & Jørgensen, E. (2021). Biofilm Research in Bovine Mastitis [Review of Biofilm Research in Bovine Mastitis]. Frontiers in Veterinary Science, 8. Frontiers Media. https://doi.org/10.3389/fvets.2021.656810

Ramesh, V., Sivakumar, R., Annamanedi, M., Chandrapriya, S., Isloor, S., Rajendhran, J., & Hegde, N. R. (2024a). Genome sequencing and comparative genomic analysis of bovine mastitis-associated non-aureus staphylococci and mammaliicocci (NASM) strains from India. Research Square (Research Square). https://doi.org/10.21203/rs.3.rs-4508846/v1

Ramesh, V., Sivakumar, R., Annamanedi, M., Chandrapriya, S., Isloor, S., Rajendhran, J., & Hegde, N. R. (2024b). Genome sequencing and comparative genomic analysis of bovine mastitis-associated non-aureus staphylococci and mammaliicocci (NASM) strains from India. Scientific Reports,

14(1). https://doi.org/10.1038/s41598-024-80533-9

Ramuada, M., Tyasi, T. L., Gumede, L., & Chitura, T. (2024). A practical guide to diagnosing bovine mastitis: a review. Frontiers in Animal Science, 5. https://doi.org/10.3389/fanim.2024.1504873

Rediger, D., Butty, M. A., Kittl, S., Bodmer, M., & Hartnack, S. (2022). Bayesian latent class models to determine diagnostic sensitivities and specificities of two point of care rapid tests (Selma plus, Dipslide) for the detection of Streptococcus uberis associated with mastitis in dairy cows. Frontiers in Veterinary Science, 9. https://doi.org/10.3389/fvets.2022.1062056

Robi, D. T., Mossie, T., & Temteme, S. (2024). A Comprehensive Review of the Common Bacterial Infections in Dairy Calves and Advanced Strategies for Health Management. Veterinary Medicine Research and Reports, 1. https://doi.org/10.2147/vmrr.s452925

Romero, R. A. R., & Vargas-Bello-Pérez, E. (2023). Non-aureus staphylococci and mammaliicocci as a cause of mastitis in domestic ruminants: current knowledge, advances, biomedical applications, and future perspectives – a systematic review [Review of Non-aureus staphylococci and mammaliicocci as a cause of mastitis in domestic ruminants: current knowledge, advances, biomedical applications, and future perspectives – a systematic review]. Veterinary Research Communications, 47(3), 1067. Springer Science+Business Media. https://doi.org/10.1007/s11259-023-10090-5

Rowe, S., House, J. K., Pooley, H. B., Bullen, S., Humphris, M., Ingenhoff, L., Norris, J. M., & Zadoks, R. N. (2024). Evaluation of point-of-care tests for identification of pathogens to inform clinical mastitis treatment decisions in pasture- and confinement-managed dairy cows in Australia. Journal of Dairy Science, 107(10), 8271. https://doi.org/10.3168/jds.2023-24612

Saeed, S. I., Kamaruzzaman, N. F., Gahamanyi, N., Nguyen, T. T. H., Hossain, D., & Kahwa, I. (2024). Confronting the complexities of antimicrobial management for Staphylococcus aureus causing bovine mastitis: an innovative paradigm. Irish Veterinary Journal, 77(1). https://doi.org/10.1186/s13620-024-00264-1

Saeed, S. I., Yazid, K. A. M., Hashimy, H. A., Dzulkifli, S. K., Nordin, F., Him, N. A. N., Omar, M. F. F. bin, Aklilu, E.,

Mohamad, M., Zalati, C. W. S. C. W., & Kamaruzzaman, N. F. (2022). Prevalence, Antimicrobial Resistance, and Characterization of Staphylococcus aureus Isolated from Subclinical Bovine Mastitis in East Coast Malaysia. Animals, 12(13), 1680. https://doi.org/10.3390/ani12131680

Salat, O., Lemaire, G., Durel, L., & Perrot, F. (2023). Etiology of severe mastitis in French dairy herds. PLoS ONE, 18(12). https://doi.org/10.1371/journal.pone.0295614

Schuler, A. M., Rice, C., & Gordon, P. J. (2017). Survey of treatment practices on Midwest dairy farms. The Bovine Practitioner, 190. https://doi.org/10.21423/bovine-vol51no2p190-199

Souza, M. M. S. de, Dubenczuk, F. C., Melo, D. A. de, Holmström, T. C. N., Mendes, M. B., Reinoso, E., Coelho, S. M. O., & Coelho, I. S. (2024). Antimicrobial therapy approaches in the mastitis control driven by one health insights. Brazilian Journal of Veterinary Medicine/Revista Brasileira de Medicina Veterinária, 46. https://doi.org/10.29374/2527-2179.bjvm002624

Speksnijder, D. C., Verduijn, H. C., Haren, S. van, Ussing, T., & Werven, T. van. (2024). Laboratory evaluation of a rapid diagnostic test for dairy mastitis. Journal of Dairy Research, 91(1), 67. https://doi.org/10.1017/s0022029924000104

Tiwari, J. G. (2013). Trends Intherapeutic and Prevention Strategies for Management of Bovine Mastitis: An Overview. Journal of Vaccines & Vaccination, 4(2). https://doi.org/10.4172/2157-7560.1000176

Touaitia, R., Ibrahim, N. A., Touati, A., & Idres, T. (2025). Staphylococcus aureus in Bovine Mastitis: A Narrative Review of Prevalence, Antimicrobial Resistance, and Advances in Detection Strategies. Antibiotics, 14(8), 810. https://doi.org/10.3390/antibiotics14080810

Wapenaar, W., Archer, S. C., Remnant, J., & Murphy, A. (2017). Control of infectious diseases in dairy cattle. In Burleigh Dodds series in agricultural science (p. 457). Burleigh Dodds Science Publishing Limited. https://doi.org/10.19103/as.2016.0006.23

Wilm, J., Svennesen, L., Kirkeby, C., & Krömker, V. (2024). Treatment of clinically severe bovine mastitis – a scoping review. Frontiers in Veterinary Science, 11, 1286461. https://doi.org/10.3389/fvets.2024.1286461