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Bovine Clinical Mastitis: From Etiology and Pathogenesis to Diagnostic Advances and Sustainable Treatment Strategies

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Abstract

Clinical mastitis remains a major economic and welfare challenge in dairy cattle, causing significant milk yield losses, reduced milk quality, increased treatment and culling costs, as well as potential zoonotic transmission of pathogens like Staphylococcus aureus and antimicrobial residues in milk. This review synthesizes current knowledge on the etiology, pathogenesis, diagnosis, and treatment of clinical mastitis, drawing from recent studies. Contagious pathogens (e.g., Staphylococcus aureus, Streptococcus agalactiae) and environmental bacteria (e.g., Escherichia coli, Klebsiella spp.) drive infections, with pathogenesis involving bacterial invasion and inflammatory immune responses. Diagnostic methods include bacterial culture, California Mastitis Test (CMT), PCR, ELISA, and emerging nanotechnology-based biomarkers for rapid, on-farm pathogen detection. Treatment strategies focus on judicious antimicrobial use, with intramammary and systemic therapies tailored to Gram-positive and Gram-negative pathogens, complemented by anti-inflammatory agents and supportive care. Alternative approaches, such as bacteriophage therapy, phytotherapy (e.g., oregano and thyme essential oils), and animal-derived compounds like propolis and lactoferrin, show promise in reducing antibiotic reliance. The review emphasizes the importance of selective dry cow therapy, enhanced hygiene practices, and integrated management protocols to improve cure rates, reduce economic losses, and ensure antimicrobial supervision for sustainable dairy production.

Keywords: clinical mastitis, dairy cattle, antimicrobial resistance, treatment strategies, bovine mastitis vaccines, One Health

INTRODUCTION

Milk and dairy products are important food sources for populations. It is estimated that around 80% (6 billion) of the world population consumes milk and dairy products (Muehlhoff and FAO, 2013). The growing global demand for dairy products has driven genetic selection to increase milk yield in cows. One of the greatest side effects of genetic selection toward milk production is increased udder health problems,

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particularly mastitis (Oltenacu and Broom, 2010). Mastitis remains a major concern in dairy production due to its adverse effects on milk yield, quality, and animal welfare. Clinical mastitis is primarily a result of bacterial invasion of the mammary gland. These pathogens are commonly classified as either contagious or environmental. Contagious pathogens include organisms that reside in the udder or teat skin and spread from cow to cow during milking. Environmental pathogens, in contrast, are found in the cow's surroundings and can infect the mammary gland between milkings or during the dry period (Roland Meçaj et al., 2023, Watts, 1988).

Mastitis is a risk for consumer health, zoonotic agents can be transmitted to them through consuming contaminated milk or direct contact with the animals. In addition, animals affected with mastitis are a source of infection for other individuals, within and outside the farm (Caneschi et al., 2023).

It accounts for significant economic losses worldwide, with estimates suggesting that the cost per animal affected by mastitis is €485 (Heikkilä et al., 2012). In addition to reduced productivity, treatment costs, and discarded milk, mastitis also requires increased labor and culling of affected animals (Li et al., 2023).

Antibiotics have been widely used to treat mastitis, but indiscriminate use has increased concerns about antibiotic resistance. Thus, professionals and researchers are looking for alternative treatment methods. Recent reports demonstrate an increase in resistance to major antibiotic classes among pathogens causing bovine mastitis (Morales-Ubaldo et al., 2023). Inappropriate use of antibiotics may increase the population of resistant bacteria in both animal and human populations (Padol et al., 2015). Additionally, in most cases, treatment begins before bacterial identification. Mastitis treatment based on bacterial isolation results in a reduction of 25 to 50% in antibiotic use, without affecting efficiency (Lago and Godden, 2018, McDougall et al., 2018). Moreover, specific treatment reduces discarding of milk and potential drug residues in products (Neeser et al., 2006). The multifactorial nature of clinical mastitis, involving host, environmental, and pathogen-related factors, poses challenges for effective management. As global concerns over antimicrobial resistance intensify, there is growing pressure to minimize the indiscriminate use of antibiotics in livestock. Consequently, the dairy industry is transitioning toward more selective and sustainable mastitis management protocols (Kour et al., 2023).

review is structured as follows: Following this introduction, it explores the etiology and pathogenesis of clinical mastitis, including key pathogens and mechanisms of infection. It then examines diagnostic approaches, ranging from clinical signs and traditional laboratory methods to emerging on-farm and nanotechnology-based techniques. Next, it addresses management and treatment strategies, emphasizing preventive measures, antimicrobial stewardship, conventional therapies tailored to Gram-positive and Gram-negative pathogens, supportive care, and treatment protocols. It also covers emerging and alternative therapies, such as bacteriophage therapy, phytotherapy, animal-derived compounds, and nanotechnology. Finally, it synthesizes the key findings in a discussion and conclusions section, highlighting research gaps, practical implications for dairy production, and future directions for sustainable mastitis control.

METHODS

In compiling this in-depth review, we systematically searched electronic databases to identify the most relevant peer-reviewed articles, reports, and book chapters on clinical mastitis in dairy cattle. We focused on key areas such as its etiology, pathogenesis, diagnostic methods, treatment options, antimicrobial stewardship, and promising alternative therapies.

Our primary databases were PubMed, Web of Science, Scopus, and CAB Abstracts. We conducted searches from January 2013 to July 2025 to capture the latest developments, while incorporating the oldest references cited. Additionally, we examined the reference lists of prominent articles—such as reviews from the *Journal of Dairy Science* and *Frontiers in Veterinary Science*—and reports from organizations like the FAO to identify additional sources.

We developed our search terms using Boolean operators such as AND and OR to connect them effectively. Examples include: ("bovine mastitis" OR "clinical mastitis" OR "dairy cattle mastitis") AND ("etiology" OR "pathogenesis" OR "diagnosis" OR "treatment" OR "management" OR "antimicrobial resistance" OR "antimicrobial stewardship" OR "alternative therapies" OR "bacteriophage therapy" OR "phytotherapy" OR "propolis" OR "lactoferrin" OR "nanotechnology" OR "preventive measures" OR "dry cow therapy"). To broaden the search, we employed truncation (e.g., mastit*) and synonyms (e.g., "udder health" or "intramammary infection"). We imposed no initial language restrictions, but for full-text review, we limited ourselves to English publications due to resource constraints.

Regarding inclusion criteria, we selected original research, reviews, and meta-analyses from peer-reviewed journals or reputable outlets. These had to address bacterial pathogens (e.g., Staphylococcus aureus or Escherichia coli), diagnostic tools (e.g., PCR or CMT), traditional and alternative treatments, economic impacts, and regulations such as EU Regulation 2019/6. We excluded materials focused solely on subclinical mastitis, non-bovine animals, or non-peer-reviewed sources (e.g., blogs or conference abstracts without full papers).

After screening titles, abstracts, and full texts for relevance and quality, we selected 99 references. We prioritized high-quality evidence from randomized controlled trials, field studies, and systematic reviews where available. We synthesized the information in a narrative format, using tables to compare pathogens and outline treatment protocols. Given the broad scope of the review, we did not perform a formal meta-analysis.

ETIOLOGY AND PATHOGENESIS

Etiology. Contagious mastitis pathogens such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma bovis* are transmitted mainly during milking. These organisms often lead to chronic infections that are difficult to eradicate. *Staphylococcus aureus*, in particular, is known for forming micro-abscesses and evading the host immune system by residing within mammary epithelial cells, which limits the efficacy of many antibiotics (Barkema et al., 2006). Table 1.

Environmental mastitis pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Streptococcus uberis* originate from bedding materials, manure, and other components of the farm environment (Roland Meçaj et al., 2023). Unlike contagious pathogens, environmental bacteria can infect cows at any time, with infections often peaking during the dry period and early lactation (20-30% higher) (Caneschi et al., 2023). Acute and severe forms of clinical mastitis are commonly associated with Gram-negative bacteria like *Escherichia coli* (Oliveira and Ruegg, 2014). Table 1.

Pathogenesis. The pathogenesis of clinical mastitis begins when pathogens penetrate the teat canal and enter the mammary gland. They then multiply and trigger a local immune response characterized by the recruitment of polymorphonuclear neutrophils (PMNs), release of pro-inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α), and an increase in somatic cell count (SCC). Virulence factors, such as *Escherichia coli* lipopolysaccharides (LPS) for endotoxemia and biofilms in *Staphylococcus aureus*, contribute to persistence and severity (Oliveira et al., 2011, Rainard et al., 2018). The ensuing inflammation results in the classic signs of mastitis: swelling, heat, redness, pain, and abnormal milk (Barkema et al., 2006).

Table 1. Common Pathogens Associated with Clinical Mastitis

Pathogen	Type	Clinical Characteristics
Staphylococcus aureus	Contagious	Chronic, subclinical or mild clinical
Streptococcus agalactiae	Contagious	Mild to moderate clinical
Streptococcus uberis	Environmental	Mild to severe, Biofilm formation
Escherichia coli	Environmental	Acute, often severe
***Klebsiella* spp.**	Environmental	Acute, gangrenous in some cases
***Mycoplasma* spp.**	Contagious	Multiquarter involvement, poor response

DIAGNOSIS

Clinical Signs

Diagnosis begins with clinical observation, including examining milk for abnormalities and assessing udder health for signs of inflammation or pain. The characteristic signs of clinical mastitis include:

1. Milk abnormalities (flakes, clots, watery consistency)
2. Swelling, redness, and heat of affected quarter
3. Decreased milk yield
4. Systemic signs in severe cases: fever, lethargy, anorexia

Clinical signs can be defined based on severity as mild, moderate and severe clinical mastitis. Mild signs are milk abnormalities manifested as clots, flakes, and/or changes in color and consistency of the milk secretion. Moderate signs are abnormalities in milk and the mammary gland (with inflammatory changes such as redness, edema, pain during palpation, and local high temperature). Severe signs are: abnormality in milk and udder gland, the animal manifest systemic clinical signs (with fever, reduction of rumination rate, reduction of appetite, lethargy, dehydration etc.,) (Adkins and Middleton, 2018).

Figure 1. Acute clinical mastitis in Holstein Friesian.

Given these observable indicators, confirmatory diagnostics emphasize pathogen identification, inflammation assessment (e.g., SCC), and susceptibility testing to guide targeted treatment. High SCC (>200,000 cells/mL) signals infection, while >2,000,000 cells/mL suggests chronic cases with poor prognosis (Algharib et al., 2024). Rapid, on-farm tools promote selective therapy, minimizing antibiotic overuse and supporting stewardship. Mastitis diagnosis measures inflammatory responses, while intramammary infection confirmation relies on agent isolation. Automated systems, like in-line SCC monitors and biosensors, enable real-time detection in modern farms.

Classic Laboratory Methods

Traditional lab techniques focus on direct pathogen identification, with bacterial culture as the cornerstone, complemented by molecular assays.

Bacterial Culture

Bacterial culture remains the gold standard for identifying mastitis-causing microorganisms, using culture media, colony morphology, staining, and biochemical tests (e.g., hemolysis, citrate utilization, KOH, CAMP) (Martins et al., 2019). Most pathogens grow aerobically, though some (e.g., *Mycoplasma* spp.) require specialized conditions. Sensitivity is limited, detecting ≥100 CFU/mL in 0.01 mL milk samples. Advantages include low cost. Drawbacks are time (24–48 hours), need for standardization, and skilled personnel (Algharib et al., 2024).

Molecular and Immunological Methods

These provide faster, more sensitive alternatives to culture, targeting DNA or antigens.

Polymerase Chain Reaction (PCR)

PCR amplifies pathogen DNA with high sensitivity/specificity (~80–95% due to inhibitors), positioning it as an emerging gold standard (Chakraborty et al., 2019). Variants include conventional (DNA fragment amplification), real-time (RT-PCR; quantification), and multiplex (multi-pathogen detection), targeting genes like 16S/23S rRNA (minimum 10^2 CFU/mL). Commercial RT-PCR kits offer rapid results, but limitations include false positives from contaminants or inhibitors in milk. Proper sampling is critical. Recent advancements integrate PCR with loop-mediated isothermal amplification (LAMP), fluorescence in situ hybridization (FISH), and genomics/proteomics for quicker, field-applicable detection (Duarte et al., 2015). Table 2.

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA detects antigens or inflammation markers (e.g., haptoglobin) via antibody-antigen binding and colorimetric signals (Kour et al., 2023). Indirect ELISA identifies pathogens like *Mycoplasma* spp. and *Streptococcus agalactiae*. Innovations like digital/nano/aptamer-based ELISA enhance sensitivity for multi-protein targeting (Chakraborty et al., 2019). Table 2.

On-Farm Tests

These enable immediate decisions without labs, prioritizing speed and practicality.

On-Farm Bacteriological Identification

Using simple media (e.g., MacConkey/blood agar) or bi/tri-plates, this method categorizes Gram-positive/negative pathogens. It supports timely treatment but lacks species precision and misses some agents (Lam et al., 2009).

Electrical Conductivity

It measures ion leakage (Na^+/Cl^-) in milk and is automated for quick results (70–90% sensitivity/specificity). It is best on foremilk. Limitations include early-stage false negatives and cost for small farms (Narváez-Semanate et al., 2022). Systems like VINTEK2 use fluorescence for bacterial detection (Algharib et al., 2024).

California Mastitis Test (CMT)

It provides indirect SCC estimation via gel reaction, with ~82% sensitivity/81% specificity for major pathogens, dropping to 61% for minor ones (Stanek et al., 2024). Cost-effective (€0.04/test) for routine screening (Kour et al., 2023). Table 2.

Lateral Flow Immunoassay

It uses antigen-antibody visualization for pathogens (94% sensitivity/81% specificity). It requires 6-hour preincubation and detects $\geq 10^3$ CFU/mL in 6 minutes (Duarte et al., 2015).

Emerging Technologies

Innovations enhance speed, specificity, and on-farm utility.

Nanotechnology

Nanoparticle-based assays (e.g., gold/magnetic conjugates) detect pathogens like *Staphylococcus aureus* via fluorescence in <3 hours (Tommasoni et al., 2023). Challenges include false negatives from clots or variability. Nanobiosensors show promise for early detection (Chakraborty et al., 2019).

Biomarkers

Elevated proteins like serum amyloid A (SAA; more accurate in milk) and bovine serum albumin (BSA) indicate inflammation (Giagu et al., 2022). They are measured via immunodiffusion and are higher in infected quarters. Proteomics/genomics uncover new biomarkers for sensitive assays (Tommasoni et al., 2023).

Artificial Intelligence (AI) and Machine Learning

AI models classify mastitis using milk properties (e.g., protein, fat, pH, lactose) with ~89% accuracy (Tommasoni et al., 2023). Adaptive neuro-fuzzy inference systems (ANFIS) improve prediction in Holstein Friesian cattle. Trends include AI for real-time monitoring and integration with genomics (Duarte et al., 2015).

Table 2. Comparison of Diagnostic Methods for Clinical Mastitis.

Method	Sensitivity (%)	Specificity (%)	Time	Cost	Advantages	Disadvantages
Bacterial Culture	70–90	90–95	24–48h	Low	Gold standard, identifies species	Time-consuming, requires lab
PCR	90–100	95–100	2–6h	Medium	Rapid, multiplex	Inhibitors in milk, cost
ELISA	80–95	85–95	1–4h	Medium	Detects biomarkers	Less sensitive for minor pathogens
CMT	60–82	70–81	Minutes	Very low	On-farm, cheap	Indirect, pathogen-agnostic
Electrical Conductivity	70–90	70–90	Minutes	Low-Medium	Fast, automated	False negatives early, no specificity
Lateral Flow Immunoassay	94	81	6 min (post-incubation)	Low	Field-friendly, cost-effective	Requires preincubation
Nanotechnology	85–95 (emerging)	90–100	<3h	High	Rapid, specific	False negatives from clots, expensive
Biomarkers (e.g., SAA)	80–95	85–95	1–4h	Medium	Indicates severity	Confounded by stress
AI/ML Models	85–95	80–90	Real-time	Variable	Predictive, integrates data	Requires training data, emerging

MANAGEMENT AND TREATMENT STRATEGIES

The control of mastitis is principally based on preventive measures and antibiotic therapy (Stanek et al., 2024, Zigo et al., 2021). In recent years, alternative treatment approaches based on natural compounds, such as propolis, have emerged as potential options to antibiotics (Ajose et al., 2022). Efficient mastitis control requires early diagnosis using on-farm rapid tests like the CMT or newer methods. This is along with identification of the causative agent to understand pathogenesis and adopt new preventive strategies that minimize transmission to healthy individuals (Tommasoni et al., 2023). Preventive measures primarily focus on milking time, when the teat canal and udder are most vulnerable to infections, or during the dry-off period (Stanek et al., 2024, Zigo et al., 2021). Teat disinfection before and immediately after milking is one of the key preventive measures (Zigo et al., 2021). Current efforts emphasize prevention to reduce mastitis prevalence in dairy cattle (Stanek et al., 2024). All preventive and control strategies must include careful, monitored antibiotic use to avoid residues and resistance in milk (More et al., 2022). The indiscriminate use of antibiotics has raised significant concerns about antimicrobial resistance (Ajose et al., 2022). Currently, veterinary authorities and practitioners are striving to minimize antibiotic use by applying alternative approaches or reserving them for indispensable cases where all other options have been considered (Preine et al., 2022, Simjee and Ippolito, 2022). The new Veterinary Medicinal Products Regulation (EU) 2019/6, effective from January 2022, also aims to reduce antibiotic use in mastitis treatment for dairy cattle in the European Union (Preine et al., 2022, Schmerold et al., 2023, Simjee and Ippolito, 2022).
This directive introduces key changes, including:

1. Selective dry cow therapy (SDCT), where only specific individuals are treated before entering the dry period based on risk assessment (including SCC, previous mastitis history, and bacterial culture), rather than blanket treatment for all animals (More et al., 2022).
2. Restrictions on the application of Highest Priority Critically Important Antimicrobials (HP-CIAs) only in cases where bacterial culture and antibiotic susceptibility testing indicate no other effective alternatives (Schmerold et al., 2023, Simjee and Ippolito, 2022).
3. Emphasis on training and education for farmers and veterinarians through programs on new protocols, risk assessment techniques, proper bacterial culturing, and antibiotic stewardship to ensure effective implementation of SDCT and restricted antibiotic use, thereby reducing unnecessary applications (More et al., 2022).

Preventive Measures and Antimicrobial Stewardship

The principal Gram-negative bacterial pathogens causing clinical mastitis are *Escherichia coli* and *Klebsiella* spp., both environmental pathogens found in the housing environment of dairy cattle (Cheng and Han, 2020, Massé et al., 2020). *Escherichia coli* generally causes acute mastitis, often of high severity, leading to irreversible mammary gland tissue damage, complete milk loss, and in severe cases animal death due to endotoxemia from lipopolysaccharides. The severity depends on the animal's immune response and lactation stage (Burvenich et al., 2003, Stanek et al., 2024). Effective antimicrobials listed in Table 3, such as fluoroquinolones and cephalosporins, are restricted to cases confirmed by bacterial isolation and susceptibility testing, aligning with antibiotic stewardship guidelines (Schukken et al., 2011, Suojala et al., 2013). Antibiotics are recommended for acute *Escherichia coli* mastitis during the postpartum period (Suojala et al., 2010), but should be avoided in mild or moderate cases to reduce resistance risks (Suojala et al., 2013, 2010). In contrast, *Klebsiella* spp. cause severe or asymptomatic mastitis, often with prolonged milk loss post-infection due to their opportunistic nature (Azwai et al., 2024, Massé et al., 2020). These bacteria frequently exhibit antimicrobial resistance, particularly to β-lactamases, leading to poor treatment outcomes (Azwai et al., 2024, Schukken et al., 2011). *Klebsiella pneumoniae* can also pose a rare zoonotic risk to humans through direct contact or contaminated environments (Fu et al., 2022). Antibiotics remain the cornerstone of mastitis treatment. Intramammary therapy is typically used for mild-to-moderate cases, while severe cases require systemic antibiotics.

Various Gram-positive bacteria cause clinical mastitis in dairy cattle, with the most predominant being *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis* (Li et al., 2023). In contrast to Gram-negative pathogens, which often lead to acute and severe infections, Gram-positive pathogens typically produce more persistent infections that are mild to moderate in severity, necessitating tailored treatment strategies. However, severe clinical forms caused by Gram-positive bacteria are not excluded (Cheng and Han, 2020). *Staphylococcus aureus* is the predominant bacterium associated with udder infections in cattle. As a contagious pathogen, it is typically transmitted from cow to cow during the milking process (Naranjo-Lucena and Slowey, 2023). Infections from *Staphylococcus aureus* are generally associated with subclinical and clinical forms of mastitis. While it triggers an immune response, *Staphylococcus aureus* often evades effective host immunity through mechanisms like intracellular persistence. Treatment of *Staphylococcus aureus* infections typically involves antibiotics, as detailed in Table 4. However, antibiotic choice must be made carefully, as some strains are methicillin-resistant and do not respond to treatments with ß-lactam antibiotics. Recent studies highlight ongoing antimicrobial resistance in bovine milk samples (Rainard et al., 2018, Winther et al., 2024). Additionally, *Staphylococcus aureus* produces biofilms that make it difficult for antibiotics to reach the bacteria (Oliveira et al., 2011). *Streptococcus agalactiae* is a Gram-positive pathogen that causes contagious mastitis. It can survive for extended periods in the cattle mammary gland by forming biofilms, which also make it difficult to be targeted by antibiotics (Cheng and Han, 2020). Penicillin and cephalosporins are among the most effective antibiotics against *Streptococcus agalactiae*, though resistance monitoring is essential [66]. *Streptococcus dysgalactiae* is another contagious Gram-positive pathogen, often transmitted during milking, and can cause both subclinical and clinical mastitis. It responds well to ß-lactam antibiotics like penicillin, but biofilms may reduce efficacy in chronic cases (Souza et al., 2024). *Streptococcus uberis* is an environmental pathogen. ß-lactams, specifically penicillin, are the most effective antibiotics against it (Zouharova et al., 2023). Table 3.

Table 3. Treatment of Clinical mastitis caused by Gram-negative bacteria.

Bacteria	Type of Infection	Intramammary Treatment	Systemic Treatment	Supportive Therapy	Animals Treated	Animals Fully Recovered	Notes	Reference
Escherichia coli	Natural	kanamycin sulfate (300 mg/cow/day) and penicillin-G-procaine (300,000 U/cow/day)	kanamycin sulfate (4,000–6,000 mg/cow/day)	IV: 7.2% sodium chloride (2,000 ml/cow/day), heparin sodium 1,000 U (25–50 ml/cow/day), physiological saline (2,000–8,000 ml/cow/day), 5% glucose (2,000–5,000 ml/cow/day)	24	17	All cows treated, 17 survivors (recovered), 7 non-survivors (died or euthanized).	(Hagiwara et al., 2014)
Escherichia coli	Induced	-	5 mg enrofloxacin/kg body weight	-	6	-	Accelerated bacterial clearance; reduced severity of local signs.	(Hoeben et al., 2000)
Escherichia coli	Natural	-	5 mg/kg Body Weight (BW) twice (24h interval): first IV, second SC.	Ketoprofen 3 mg/kg BW IM.	66	-	Differences between treated and non-treated group were slight (clinical cure ~46.7%).	(Suojala et al., 2010)
Escherichia coli	Natural	-	Ceftiofur 2.2 mg/kg IM, repeated every 24h for five doses	-	51	47	Reduced the proportion of cow death or culling	(Erskine et al., 2002)
Klebsiella pneumoniae	Natural	-	Cefazolin 5.0 mg/kg in 1L saline, IV infusion over 1hr every 24hr from first to final visit. If no improvement by day 2, switch to fluoroquinolone	-	208	-	When fluoroquinolone applied on the second day, resulting in cure rates rise from 52.8% to 76.7% for *K. pneumoniae*.	(Sugiyama et al., 2022)

			(enrofloxacin 5 mg/kg IV once daily or orbifloxacin 5 mg/kg IM once daily).					
Klebsiella pneumoniae	Natural	Intramammary infusions once daily for 4d: ceftiofur hydrochloride 125 mg	-	-	13	4		(Schukken et al., 2011)

Table 4. Treatment of Clinical mastitis caused by Gram-positive bacteria.

Pathogen	Naturally Occurring Infection	Intramammary Treatment	Systemic Treatment	Supportive Therapy	Treated Animals	Cured Animals	Notes	Reference
Staphylococcus aureus	Yes	N/A	Tylosin (10 mg/kg SC) and marbofloxacin (8 mg/kg SC) for 3 days, 21 days before calving	N/A	291 (TYLO), 275 (MARB)	TYLO (3.8%), MARB (5.8%)	Preventive	(Amiri et al., 2024)
Staphylococcus aureus	No (field study, not specified)	Ceftiofur hydrochloride (500 mg/10 mL intramammary) and enrofloxacin (300 mg/10 mL intramammary) for 5 days	N/A	N/A	14	9	80% cure rate	(Alfonseca-Silva et al., 2021)
Streptococcus agalactiae	No (in vitro)	N/A	Aminoglycosides (kanamycin, gentamicin, neomycin, tobramycin); in vitro (China)	N/A	N/A	N/A	In vitro susceptibility	(Han et al., 2022)
Streptococcus agalactiae	No (in vitro)	N/A	Penicillin; in vitro (Sweden)	N/A	N/A	N/A	In vitro susceptibility	(Persson et al., 2011)
Streptococcus uberis	No (field study, not specified)	N/A	Amoxicillin (15 mg/kg IM every other day for 3 days)	N/A	30	24	Clinical cure in trial	(Hillerton and Kliem, 2002)

Procaine benzylpenicillin and amoxicillin are first-line for Gram-positive infections (Svennesen et al., 2023). Ceftiofur and cefquinome are effective against coliform bacteria (Erskine et al., 2002). Tylosin and florfenicol are for *Mycoplasma* spp. and penicillin-resistant strains (Gelgie et al., 2024). Antibiotic selection should be guided by culture and sensitivity to reduce resistance. Blanket antibiotic use without diagnostics can lead to treatment failure, residue violations, and selection for resistant strains.

Vaccination

Vaccination is a key preventive strategy to reduce the incidence and severity of clinical mastitis, particularly for common pathogens like *Staphylococcus aureus*, *Streptococcus* spp., and *Escherichia coli* (Rainard et al., 2021). Vaccines stimulate the cow's immune system to produce antibodies and enhance cellular immunity, reducing infection severity and duration. Commercially available vaccines, such as those targeting *Staphylococcus aureus* (e.g., Startvac, Hipramun), combine antigens from *Staphylococcus aureus*, *Streptococcus uberis*, and *Escherichia coli* to provide broad protection (Rainard et al., 2021). Field studies show vaccination reduces mastitis incidence by 20–40% and improves cure rates, though efficacy varies with pathogen strain and farm conditions (Mata et al., 2023). For example, *Escherichia coli* vaccines targeting J5 strains decrease coliform mastitis severity by enhancing opsonization and phagocytosis (Wilson et al., 2007). Challenges include variable immune responses due to cow genetics and pathogen diversity, and vaccines are less effective against chronic infections like *Staphylococcus aureus* due to its intracellular persistence (Kerro Dego and Vidlund, 2024, Rainard et al., 2022). Vaccination programs are most effective when integrated with hygiene and SDCT, administered pre-calving or during the dry period to boost immunity before high-risk periods. Ongoing research aims to develop multivalent vaccines targeting emerging resistant strains (Kerro Dego and Vidlund, 2024).

Supportive Therapy

Supportive treatment is essential, especially in acute or systemic cases:
NSAIDs (e.g., flunixin meglumine) reduce inflammation and fever (Green et al., 1997, Wilm et al., 2024).
Fluids may be necessary to combat dehydration and shock (Green et al., 1997).
Oxytocin can assist with milk letdown and complete emptying of the infected quarter (Green et al., 1997).

Treatment Protocols

Protocols vary by severity:
Mild: Intramammary antibiotics for 3–5 days.
Moderate: Combination of systemic and intramammary antibiotics.
Severe: Intensive therapy including fluids, anti-inflammatories, and culture-based antibiotics.
Recurrence is common especially with, *Staphylococcus aureus*, which forms intracellular reservoirs and biofilms that resist antibiotics. Extended therapy and dry cow treatment may be needed in these cases (Barkema et al., 2006).

EMERGING AND ALTERNATIVE THERAPIES

Bacteriophage Therapy

Bacteriophages are viruses that selectively infect bacteria, causing their lysis. In recent years, bacteriophage therapy has been viewed as a promising alternative to antibiotics (Cheng and Han, 2020). Bacteriophages are characterized by high specificity and low toxicity (Saeed et al., 2024). The main disadvantage reported for bacteriophages is the host immune system's reaction, which decreases their activity against a specific pathogen (Brouillette et al., 2023). Several experiments using mouse models have demonstrated that bacteriophage therapy is valid for treating udder infections (Brouillette et al., 2023, Teng et al., 2022). During these studies, a disadvantage was an increase in SCC in healthy quarters (Brouillette et al., 2023). A bacteriophage cocktail (SYGD1, SYGE1, and SYGMH1) was effective against mastitis caused by *Escherichia coli*, reducing bacterial counts, SCC, and mastitis symptoms in cattle, with an effect similar to that of antibiotics (Guo et al., 2021). Regarding *Staphylococcus aureus*, a bacteriophage cocktail (vBSM-A1 and vBSP-A2) was effective in reducing mastitis inflammation in mice (Geng et al., 2020).In a study conducted by Mohammadian *et al.*(Mohammadian et al., 2022), a combination of *Staphylococcus* phages B4 and B8 was effective against methicillin- and multidrug-resistant *Staphylococcus aureus*. The bacteriophages PlySs2 and PlySs9 have been shown to be an alternative therapy to antibiotics for bovine mastitis caused by *Streptococcus uberis* (Vander Elst et al., 2020). Against *K. pneumoniae*, bacteriophage CM8-1 was shown to be a promising therapy for mastitis in a murine model caused by this pathogen, with reductions in bacterial counts and improvements in udder inflammation (Zhao et al., 2021).

Plant based therapy

Plants have been used in traditional medicine for a long time. Given the restrictions on antibiotic use, they are gaining interest as alternatives for treating dairy cattle mastitis. Compared to antibiotic compounds, they have the advantages of not provoking resistance even after long-term exposure and exhibiting low toxicity (Cheng and Han, 2020). The medicinal value of plants is attributed to their metabolites, such as alkaloids, flavonoids, and volatile components, which have antibacterial and anti-inflammatory properties (Li et al., 2023). For example, maize whiskers were shown to significantly inhibit biofilm formation by methicillin-resistant *Staphylococcus aureus* (Shang et al., 2019). Cannabinoids from *Cannabis sativa* were found to have a bactericidal effect against methicillin-resistant *Staphylococcus aureus* at a concentration of 0.25 mg/mL and to reduce biofilm formation at 0.125 mg/mL (Roshan et al., 2024). Essential oils from *Eugenia* spp. (Myrtaceae) were also shown to reduce *Staphylococcus aureus* at a minimum inhibitory concentration (MIC) ranging from 64 to 256 µg/mL, with inhibition of biofilm formation (Da Silva et al., 2024). *Salvia officinalis*, with active multicyclic terpenoids formulated for topical use, was effective against methicillin-resistant *Staphylococcus aureus*, with an MIC ranging from 0.09 to 0.74 mg/mL and inhibition of biofilm formation (Purgato et al., 2024). An extract of essential oils from oregano, thyme, carvacrol, and thymol was shown to have bactericidal activity against more than 30 species of *Staphylococci* (Dal Pozzo et al., 2011). *Minthostachys verticillata* essential oil and limonene were effective in eliminating *Streptococcus uberis* bacteria and inhibiting their biofilm formation at concentrations ranging from 114.5 to 229 mg/mL for M. verticillata and 210 mg/mL for limonene (Montironi et al., 2016). Essential oils from aromatic plants like oregano, chamomile flower, and black seed improved udder health in Friesian cows (Salem et al., 2019). Phyto-Bomat, a combination of essential oils such as common thyme, wild thyme, oregano, and mountain savory, was as effective and more economical compared with traditional antibiotics, it was effective against *Escherichia coli*, *Streptococcus* spp., and *Staphylococcus* spp. (Kovačević et al., 2022a, 2022b). Phyto-Bomat can also be used in combination with antibiotics for treating clinical mastitis, where the combination was effective against *Streptococcus uberis* but not against *Escherichia coli* (Tomanić et al., 2023a). A combination of tea tree oil, thymol, and carvacrol (composed of oregano and thyme essential oils) was shown to have an inhibitory effect against Gram-negative bacteria isolated from clinical mastitis (Corona-Gómez et al., 2022).

Herbal gels based on extracts of propolis, alcoholic extracts of Brewers Gold and Perle hops, plum lichen, common mallow, marigold, absinthe wormwood, black poplar buds, lemon balm, and essential oils of oregano, lavender, and rosemary, administered intramammarily in a 10 mL volume, showed a decrease in the numbers of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus* spp. (Pașca et al., 2020). In an *in vitro* experiment, a herbal soap (1% extract retrieved from *Senna macranthera* roots containing active components such as physcion, chrysophanol, and emodine) was shown to inhibit *Staphylococcus aureus* growth (Inoue Andrade et al., 2015).

Animal derived compounds

Animal-derived compounds are products originating from animals. Propolis is the most well-known animal-derived product used as a treatment alternative. It is a mixture produced by honeybees from components collected from several types of plants (Tomanić et al., 2023b). Propolis has a complex composition, with identified compounds such as terpenoids, polyphenols, amino acids, and steroids. It exhibits a wide range of biological activities, among which are well-known antibacterial, antiviral, antioxidant, anti-inflammatory, and antifungal effects (Santos et al., 2020). In cattle, propolis was shown to protect bovine mammary epithelial cells from mastitis pathogens (Wang et al., 2016). Moreover, it has been used as an intramammary treatment for mastitis prevention and control (Bacic, 2016). Propolis was effective against most bacteria causing bovine mastitis, with higher efficacy against Gram-positive bacteria compared to Gram-negative ones (Bačić et al., 2016). In other experiments, bee venom, containing the active component melittin, was shown to reduce the inflammatory response in bovine mammary epithelial cells infected with lipopolysaccharide (LPS) (Jeong et al., 2017). Lactoferrin is an iron-binding glycoprotein found in milk, colostrum, saliva, and other exocrine secretions. Lactoferrin has been shown to have bacteriostatic, bactericidal, and antifungal properties (Shimazaki and Kawai, 2017). It plays a crucial role in protecting the udder against microbial infections. The main reasons for ineffective udder protection during mastitis are related to fluctuations in lactoferrin levels and its activity against specific mastitis pathogens (Shimazaki and Kawai, 2017). It has been demonstrated to have an antimicrobial effect against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus agalactiae* (Hafez et al., 2013).

Nanotechnology Nano therapy

Nanotechnology is a rapidly growing field that has found applications in various areas, such as engineering, industry, the environment, food, and medicine. Nanotechnology therapy enables drugs to be deposited and released at specific locations, overcoming some limitations of conventional drugs, one of which is antibiotic resistance (Li et al., 2023). It has started to be used in veterinary medicine for purposes such as diagnosis, prevention, therapy, animal breeding, reproduction, and disinfection (Tomanić et al., 2023b). Silver nanoparticles at a concentration of 10 μg/mL have been shown to have an antibacterial effect against *Staphylococcus aureus* (Yuan et al., 2017). Nanoparticles also improve antibiotic efficiency. for example, chitosan nanoparticles loaded with cloxacillin were shown to enhance antibiotic efficacy against *Staphylococcus aureus* and its ability to form biofilms (Eskandari et al., 2025).

DISCUSSION AND CONCLUSIONS

Clinical mastitis remains a major hurdle in dairy cattle production, impacting economics, animal welfare, and public health through milk losses, treatment costs, and zoonotic risks. This review synthesizes its bacterial etiology—primarily contagious (*Staphylococcus aureus*, *Streptococcus agalactiae*) and environmental (*Escherichia coli*, *Klebsiella* spp.) pathogens—and pathogenesis involving immune responses like cytokine release and elevated SCC. Diagnostic progress spans traditional methods (e.g., bacterial culture, CMT) to innovations like PCR, ELISA, and nanotechnology for rapid on-farm detection. Treatment prioritizes antimicrobial stewardship, with tailored therapies for Gram-positive/negative infections, supportive NSAIDs and fluids. There is a shift toward alternatives including bacteriophages, phytotherapy (e.g., oregano/thyme oils), propolis, lactoferrin, and nanoparticles to curb antibiotic use.

Antimicrobial resistance (AMR) emerges as a core challenge, driven by past overuse, with rising multi-drug resistant strains in regions like Iran (Sharifi et al., 2023), and Zimbabwe (Katsande et al., 2025). EU Regulation 2019/6's promotion of selective dry cow therapy (SDCT) has cut antibiotic use by up to 50% without raising mastitis rates in hygienic herds, (Ut et al., 2025). However, barriers like education and diagnostics persist in resource-limited areas. Key gaps include limited field trials for alternatives, where in vitro efficacy (e.g., phage cocktails) often outpaces real-world results (<50% in bovine trials) (Nale and McEwan, 2023); underexplored factors like climate change's effects on pathogen dynamics (Guzmán-Luna et al., 2022); and the need for validation of immunotherapies and AI-integrated diagnostics (Mitsunaga et al., 2024, Saleem et al., 2024).

Key gaps include limited field trials for alternatives, where *in vitro* efficacy (e.g., phage cocktails) often outpaces real-world results, (Nale and McEwan, 2023) and underexplored factors like climate change's effects on pathogen dynamics (Guzmán-Luna et al., 2022). Immunotherapies and AI-integrated diagnostics also need more validation (Mitsunaga et al., 2024, Saleem et al., 2024).

Practically, integrated programs emphasizing hygiene, nutrition, SDCT, and culture-guided treatments can reduce costs (€150-400 per case) and improve milk quality. Veterinarians are key for training and surveillance.

Future research should prioritize novel antimicrobials, optimized alternatives, genetic enhancements for immunity, and interdisciplinary One Health strategies to combat AMR and secure dairy sustainability by 2030 (Li et al., 2023).

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