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Bovine Mastitis: Why a Universal Cure Remains Elusive Despite Decades of Research Investment — A Comprehensive Review

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ABSTRACT

Bovine mastitis, defined as inflammation of the mammary parenchyma, represents the most economically significant and therapeutically challenging infectious disease in global dairy production. The disease imposes annual losses conservatively estimated at USD 19–35 billion worldwide through reduced milk yield, discarded antibiotic-contaminated milk, premature culling, veterinary expenditure, and quality premium forfeiture. It affects an estimated 25–50% of dairy cows in organized production systems and carries substantial animal welfare implications including pain, pyrexia, endotoxemia, and case-fatality rates of 5–15% in peracute coliform mastitis as well as public health risks through zoonotic pathogen transmission and antibiotic residues in milk.

Despite sustained global research investment spanning more than seven decades, no universal curative or preventive strategy has emerged. This is fundamentally because mastitis is not a single disease entity but a multifactorial inflammatory syndrome triggered by more than 150 distinct microbial species, each deploying unique virulence strategies and demanding pathogen-specific management responses. This review critically examines the etiopathogenesis of bovine mastitis, analyzing the virulence mechanisms of principal causative agents including *Staphylococcus aureus* (*S. aureus*), *Streptococcus uberis*, *Escherichia coli*, *Klebsiella spp.*, and *Mycoplasma bovis*. The host mammary immune response encompassing pattern recognition receptor signaling, neutrophil extracellular trap (NET) formation, and macrophage-mediated early defense is evaluated alongside critical modulating factors including parity, stage of lactation, genetic susceptibility, and periparturient immunosuppression.

The escalating crisis of antimicrobial resistance (AMR) is critically assessed, encompassing methicillin-resistant *S. aureus* (LA-MRSA CC398), ESBL-producing and mcr-1-harboring Enterobacteriaceae, and progressive regulatory restrictions. Current treatment modalities are evaluated with quantitative efficacy data, including culture-guided selective treatment and selective dry cow therapy (SDCT). Vaccination strategies, alternative therapeutic approaches including lytic bacteriophage cocktails, host defense peptides, probiotic mammary microbiome modulation, and phytochemical preparations and precision medicine advances, including MALDI-TOF pathogen identification, multiplex PCR/LAMP diagnostics, automated inline sensor arrays, and AI-driven

predictive models, are reviewed comprehensively. Genomic selection, CRISPR-Cas9 genome editing, and nutritional immunomodulation are evaluated as evidence-based long-term complements. We propose a prioritized research agenda for 2026 and beyond, concluding that controlling mastitis to economically and ethically acceptable thresholds through sustained, evidence-based, multimodal strategies constitutes the scientifically defensible and operationally achievable goal for the global dairy industry.

KEYWORDS: Bovine mastitis, *Staphylococcus aureus*, *Escherichia coli*, Antimicrobial resistance, Intramammary infection, Bacteriophage therapy, Vaccine, Somatic cell count, Dairy cattle, Innate immunity, Precision livestock farming, Nanoparticle drug delivery.

INTRODUCTION

Mastitis, defined as inflammation of the mammary parenchyma, represents a formidable challenge to sustainable dairy production across all geographic regions and production systems. The disease encompasses a spectrum of clinical presentations, ranging from peracute life-threatening systemic illness through acute and subacute clinical mastitis to chronic subclinical intramammary infection (IMI) detectable only through elevated somatic cell counts (SCC) or microbiological culture.[1,2] Subclinical mastitis is particularly insidious: it silently erodes milk yield, alters milk composition, compromises product quality, and predisposes quarters to clinical episodes, yet remains undetectable by routine farm observation.[3]

The economic impact of bovine mastitis is staggering and multidimensional. Annual industry-wide losses in the United States have historically been estimated at USD 1.7–2.0 billion[4], although more comprehensive contemporary accounting that incorporates lost genetic merit, premature culling, reduced productive lifespan, and the indirect costs of antimicrobial stewardship compliance suggests that the true figure substantially exceeds these estimates. Globally, conservative assessments place the annual economic burden at USD 19–35 billion, with European Union losses approximating €1.5–2.0 billion annually.[5,6] These losses originate from milk production decrements, discarded antibiotic-contaminated milk, veterinary and medicament expenses, supplementary labor, premature culling of productive animals, and reduced milk quality premiums.[3,7]

Beyond economics, mastitis carries profound animal welfare consequences. Affected animals experience pain, discomfort, pyrexia, and reduced feed intake, with severe coliform mastitis causing endotoxemia, cardiovascular compromise, and fatality rates approaching 5–15% in peracute cases.[8,9] Public health dimensions are equally important: mastitis pathogens such as *Staphylococcus aureus*, *Streptococcus agalactiae*, *Listeria monocytogenes*, and *Mycobacterium species* possess zoonotic potential, while antibiotic residues in milk from inadequately managed treatment withdrawal periods pose direct consumer safety risks and contribute to broader AMR dissemination.[10,11]

Despite sustained global investment in mastitis research spanning more than seven decades, the disease continues to afflict approximately 25–50% of dairy cows in developed production systems, with substantially higher prevalence rates in low- and middle-income countries where herd health infrastructure is limited.[12,13] This persistence reflects the biological complexity of the host-pathogen-environment triad rather than a failure of scientific endeavor. Over 150 distinct microbial species can colonize the bovine mammary gland, each deploying unique virulence strategies, occupying different ecological niches within the farm environment, and demanding pathogen-specific management responses.[1,14]

This comprehensive review synthesizes current knowledge on mastitis etiopathogenesis, evaluates the spectrum of preventive and therapeutic interventions with critical analysis of their evidence base, examines emerging technologies poised to transform mastitis management, and considers why integrated control programs rather than single-solution cures represent the scientifically rational path forward. The review incorporates the most recent literature through 2025–2026, reflecting the rapidly evolving landscape of precision livestock medicine, biotechnology, and One Health antibiotic stewardship.

ETIOPATHOGENESIS OF BOVINE MASTITIS

Classification and Causative Agents

Mastitis pathogens are conventionally classified according to their primary ecological reservoir and principal route of transmission. Contagious pathogens most notably *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma bovis* primarily reside within the infected mammary gland itself, spreading cow-to-cow during milking via contaminated milking equipment, teat liners, and milker hands.[2,15] Environmental pathogens, principally *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Streptococcus uberis*, and *Streptococcus dysgalactiae*, originate from bedding, manure, soil, and water sources, with exposure occurring during inter-milking periods when the teat canal is most vulnerable.[16,17]

Gram-positive bacteria collectively account for approximately 60–70% of IMI globally. *Staphylococcus aureus* remains the most problematic contagious pathogen owing to its sophisticated immune evasion arsenal and propensity for chronic, subclinical infection with low spontaneous cure rates.[18] Coagulase-negative staphylococci (CNS), once dismissed as minor pathogens, have emerged as the most prevalent IMI agents in well-managed herds, causing persistent subclinical mastitis with significant SCC elevation and milk quality implications.[19,20] *Streptococcus uberis* has become the predominant streptococcal mastitis pathogen in many Northern European herds following successful *S. agalactiae* eradication campaigns, with its highly diverse clonal population complicating both epidemiological tracking and vaccine development.[15]

Gram-negative pathogens, while responsible for fewer IMI on a prevalence basis, cause a disproportionate share of severe clinical mastitis cases requiring intensive intervention. *Escherichia coli* causes peracute, toxic mastitis triggered by endotoxin (lipopolysaccharide, LPS) release during rapid bacterial lysis, provoking a ferocious innate immune response that frequently exceeds the inflammatory stimulus in its destructive potential.[21,22] Klebsiella species cause mastitis with particularly high case-fatality rates in dairy cattle, and their intrinsic resistance to multiple antibiotic classes severely restricts therapeutic options.[16] Mycoplasma bovis occupies a unique pathological niche:

lacking a cell wall, it is completely refractory to all beta-lactam antibiotics, spreads silently within herds, and causes irreversible damage to mammary secretory tissue with no practical therapeutic remedy beyond culling.[23,24]

Emerging and non-bacterial pathogens have assumed increasing clinical significance in recent decades. Prototheca zopfii and Prototheca blaschkeae colorless, achlorophyllous algae cause a refractory mastitis that is universally unresponsive to antibiotic therapy; affected animals must be identified and removed from the herd to prevent intraherd spread.[25,26] Fungal mastitis caused by *Candida spp., Aspergillus spp.,* and *Trichosporon spp.* occurs most commonly following prolonged or repeated intramammary antibiotic therapy that depletes the normal microbial flora, creating a niche for opportunistic fungal colonization.[27]

Virulence Mechanisms

The capacity of bovine mastitis pathogens to establish infection, evade host defenses, and persist within the mammary gland reflects an evolutionary fine-tuning of specific virulence factors. *Staphylococcus aureus* encodes an exceptionally broad virulence repertoire, including surface-anchored proteins that mediate adhesion to host extracellular matrix components (fibronectin, fibrinogen, collagen, and vitronectin); secreted toxins (α-toxin, β-toxin, δ-toxin, and Panton-Valentine leukocidin); superantigens (staphylococcal enterotoxins A–E and toxic shock syndrome toxin-1); proteases; lipases; and immune evasion factors such as protein A, the immune evasion cluster (IEC), and chemotaxis inhibitory protein (CHIPS).[28,29]

Biofilm formation represents perhaps the most clinically consequential *S. aureus* virulence mechanism in the context of mastitis. Within mammary tissue and milk ducts, *S. aureus* forms structurally complex biofilms embedded in a polysaccharide intercellular adhesin (PIA/PNAG) matrix, reducing antibiotic efficacy by 100- to 1,000-fold relative to planktonic bacteria through multiple mechanisms: restricted antibiotic diffusion, metabolic dormancy of embedded cells, altered gene expression profiles, and enhanced horizontal transfer of resistance determinants.[30-32] Intracellular survival within bovine mammary epithelial cells and professional phagocytes further shields *S. aureus* from both immune effectors and antimicrobial agents, creating protected reservoirs from which recrudescing infections originate after apparent clinical resolution.[33,34] Recent pharmacokinetic and pharmacodynamic analyses have highlighted that therapeutic failure in chronic mastitis arises not only from classical antimicrobial resistance but also from the combined effects of biofilm-mediated tolerance, intracellular persistence, poor intramammary drug penetration, and episodic drug elimination via milk flow.[35,36]

Streptococcal virulence mechanisms differ substantially from those of *S. aureus* but are equally effective at circumventing mammary defenses. *Streptococcus uberis* expresses a hyaluronic acid capsule that inhibits phagocytosis and elaborates streptolysin, CAMP factor, and an array of surface proteins (Sua proteins) mediating adhesion to bovine plasminogen, lactoferrin, and complement component C3b.[37] Its metabolic versatility allows survival in diverse environmental matrices. *Escherichia coli* virulence in mammary tissue is mediated primarily through LPS-triggered Toll-like receptor 4 (TLR4) signalling, driving massive neutrophil influx and proinflammatory cytokine production (TNF-α, IL-1β, IL-6, IL-8) that mediates the systemic toxemia characteristic of acute coliform mastitis.[21,28]

Host Immune Response

The mammary gland immune system operates in a challenging biological context: it must protect against a diverse array of pathogens while tolerating the presence of milk and maintaining secretory function. The innate immune system constitutes the first and most critical line of defense. Teat canal anatomy the streak canal lined with stratified squamous epithelium, the keratin plug, and bacteriostatic fatty acid content of teat canal secretions provides a physical and chemical barrier against bacterial ingress.[29,39]

Once bacteria breach the teat canal, the pattern recognition receptor (PRR) system including Toll-like receptors (TLR1, TLR2, TLR4, TLR9), Nod-like receptors (NLRs), and C-type lectin receptors recognizes pathogen-associated molecular patterns (PAMPs) such as LPS, lipoteichoic acid, peptidoglycan, and bacterial DNA, triggering immediate inflammatory responses.[40,41] This innate signaling cascade activates nuclear factor-κB (NF-κB) and MAPK pathways, induces the secretion of pro-inflammatory cytokines, upregulates adhesion molecules on mammary endothelium, and orchestrates massive neutrophil transmigration from blood into milk.[39] Panigrahi et al. (2014) demonstrated that specific SNP variants within the TLR4 gene, particularly in the CRBR2 fragment, are associated with differential mastitis susceptibility in crossbred cattle[42], confirming that innate immune receptor polymorphisms modulate the magnitude and efficiency of the initial host response.[43]

Neutrophils represent the dominant cellular effectors in acute IMI, migrating into milk within hours of infection and executing pathogen elimination through oxidative burst (reactive oxygen species generation), degranulation of myeloperoxidase and elastase, phagocytosis, and neutrophil extracellular trap (NET) formation.[44,45] Macrophages resident in mammary secretory tissue assumes critical roles both in pathogen recognition during the early infection window before neutrophil influx and in resolution of inflammation following bacterial clearance. Both cell populations are functionally compromised during the periparturient period the highest-risk window for new IMI by the combined effects of hypocalcemia, negative energy balance, oxidative stress, and glucocorticoid-mediated immunosuppression.[46,47] The expression of interleukin-10 and other immunoregulatory mediators in mammary tissue further modulates the balance between pro-inflammatory and resolution-phase responses, as demonstrated in comparative studies across crossbred cattle, Tharparkar cattle, and Murrah buffalo by Sulabh et al.[48]

Individual variation in immune competence critically determines mastitis outcome. Animals with superior genetic potential for immune responsiveness demonstrate higher neutrophil chemotaxis, enhanced oxidative burst capacity, and more effective T-helper cell polarization, translating to lower IMI incidence and higher spontaneous cure rates.[21,49] This heritable variation in immune function forms the biological foundation for genetic selection

programs targeting mastitis resistance. Dige et al.[50] and Muhasin Asaf et al.[51,52] demonstrated that allelic variants at the CXCR2, MBL1, and BRCA1 loci show variable associations with mastitis susceptibility in Vrindavani crossbred cattle, underscoring the polygenic complexity of bovine mastitis resistance.

ANTIMICROBIAL RESISTANCE IN MASTITIS PATHOGENS

The emergence and global dissemination of antimicrobial resistance (AMR) among bovine mastitis pathogens constitutes one of the most significant threats to effective disease management and represents a critical One Health concern. Decades of extensive antibiotic use for therapeutic and prophylactic purposes in dairy cattle have imposed substantial selection pressure, driving the evolution and spread of resistance determinants across both commensal and pathogenic microbial populations.[53,54] The global burden of AMR in bovine mastitis pathogens encompasses a broad spectrum of resistance phenotypes and genotypes, including resistance to beta-lactams, aminoglycosides, fluoroquinolones, tetracyclines, and polymyxins, with multidrug-resistant (MDR) strains increasingly prevalent in both high-income and low- and middle-income countries.[55]

Methicillin-resistant *Staphylococcus aureus* (MRSA) carriage in dairy cattle was first reported in the 1970s and has since expanded substantially. The mecA gene, encoding the penicillin-binding protein PBP2a with dramatically reduced beta-lactam affinity, confers resistance to the entire beta-lactam antibiotic class the cornerstone of mastitis therapy rendering standard intramammary formulations ineffective.[31,56] LA-MRSA (livestock-associated MRSA, predominantly CC398) now circulates in dairy herds across Europe, North America, Asia, and Africa, with zoonotic transmission to farm workers documented in multiple countries.[57,58] A recent meta-analysis estimates the global prevalence of MRSA-associated bovine mastitis at 4.3% (95% CI: 3.24–5.50), representing a significant and growing reservoir of zoonotic risk.[35]

Extended-spectrum β-lactamase (ESBL)-producing and carbapenemase-producing Enterobacteriaceae have emerged as particularly alarming AMR threats in dairy cattle environments. ESBL-producing E. coli carrying CTX-M, SHV, or TEM enzymes have been isolated from milk, feces, and environmental samples in dairy farms across multiple continents, with resistance extending to fluoroquinolones, aminoglycosides, and tetracyclines in MDR strains.[56,59] The detection of colistin resistance mediated by the mcr-1 plasmid gene in bovine *E. coli* isolates carries particularly grave implications given colistin's critical importance as a last-resort antibiotic in human medicine.[60,61]

Regulatory responses to the AMR crisis in food-producing animals have progressively restricted therapeutic options. The European Union's implementation of Regulation 2019/6, which designates certain antimicrobial classes as reserved exclusively for human use or prohibited in food animal production, has eliminated fluoroquinolones and third/fourth-generation cephalosporins as routine mastitis treatment options in EU member states.[62] In December 2024, the United States FDA issued revised draft guidance (GFI #49) updating recommendations for evaluating the safety and effectiveness of antibacterial intramammary drug products, signaling continued regulatory evolution in this domain.[63] Comparable regulatory trajectories in Canada, Australia, and other major dairy-producing nations increasingly mandate veterinary oversight, culture-based therapy selection, and systematic antimicrobial use reporting, fundamentally transforming mastitis management practices and intensifying demand for non-antibiotic therapeutic alternatives.[64,65]

CURRENT TREATMENT APPROACHES AND THEIR LIMITATIONS

Antibiotic Therapy

Intramammary antibiotic therapy remains the cornerstone of clinical mastitis management worldwide, despite progressively accumulating evidence of modest bacteriological cure rates for many pathogen-quarter combinations. Beta-lactam antibiotics particularly penicillin-procaine, ampicillin, cloxacillin, and ceftiofur dominate intramammary formulary choices owing to their activity against prevalent Gram-positive mastitis pathogens, favorable milk residue withdrawal profiles, and regulatory approval status.[18,66] However, therapeutic failure increasingly arises from the combined effects of pharmacokinetic/pharmacodynamic (PK/PD) constraints, intramammary drug inactivation by milk components, biofilm-mediated tolerance, and intracellular persistence factors that extend well beyond classical AMR.[35,36]

Treatment efficacy demonstrates substantial pathogen-dependent variation. Bacteriological cure rates for *E. coli* mastitis approximate 70–85% given the organism's frequent spontaneous elimination by innate immune responses, while Streptococcus agalactiae cure rates of 70–90% reflect its relative antibiotic susceptibility.[18] In stark contrast, bacteriological cure rates for S. aureus IMI average only 25–40% for lactating cow therapy protocols, declining further to 15–30% in chronic infections, quarters with more than three previous clinical episodes, cows in later lactation, or animals harboring biofilm-positive strains.[18,66,67] Extended therapy protocols using systemic plus intramammary ceftiofur or prolonged intramammary cloxacillin courses modestly improve *S. aureus* cure rates to 50–65% in carefully selected first-parity animals with acute infections.[68,69]

The paradigm shift toward culture-guided therapy has substantially reduced unnecessary antibiotic use without compromising clinical outcomes. On-farm culture systems enable pathogen identification within 18–24 hours, directing withholding of antibiotic treatment for culture-negative samples, Gram-negative cases often amenable to NSAID-only therapy, and Mycoplasma-positive quarters that are unresponsive to available antibiotics.[66,70] Meta-analyses of selective treatment trials demonstrate antibiotic use reductions of 50–70% without significant detriments to clinical cure, recurrence, or milk production outcomes.[66,71]

Dry Cow Therapy

The dry period represents a critical window for both the elimination of existing IMI and the acquisition of new infections, with new infection rates during the dry period estimated at 10- to 20-fold higher per day than during lactation.[72] Dry cow therapy (DCT), encompassing intramammary antibiotic administration at

drying-off, has historically been implemented as a blanket protocol applied to all quarters of all cows. Systematic reviews and meta-analyses demonstrate that blanket DCT eliminates 70–80% of existing subclinical IMI and reduces new dry-period infections by approximately 50–70% compared to untreated controls.[72,73]

Regulatory and stewardship pressures have catalyzed a global transition toward selective dry cow therapy (SDCT), in which antibiotic infusion is reserved for quarters meeting pre-specified criteria typically SCC thresholds, culture-positive status, or previous clinical mastitis history while antibiotic-free quarters receive only internal teat sealants (ITS). The combination of SDCT and ITS has been demonstrated in multiple large-scale field trials to be non-inferior to blanket DCT in terms of new IMI prevention and clinical mastitis incidence in the subsequent lactation, while achieving antibiotic use reductions of 40–75%.[74-76] Internal teat sealants containing bismuth subnitrate in a mineral oil base form a physical seal occluding the teat canal throughout the dry period, providing protection equivalent to antibiotics against new Gram-negative IMI.[77]

Supportive and Symptomatic Therapy

Non-steroidal anti-inflammatory drugs (NSAIDs) occupy an increasingly important role in mastitis therapy as adjuncts to antimicrobial treatment and, in culture-negative or Gram-negative cases, as the primary therapeutic intervention. Flunixin meglumine and meloxicam inhibit cyclooxygenase (COX) enzymes, reducing prostaglandin synthesis, attenuating fever, and ameliorating the systemic inflammatory response syndrome (SIRS) features of acute and peracute mastitis.[9,78] Randomized controlled trial evidence supports NSAID use improving milk production recovery, reducing culling rates, and enhancing welfare outcomes in moderate-to-severe clinical mastitis, without demonstrable adverse effects on bacteriological cure when administered alongside appropriate antibiotic therapy.[9,79] Intravenous fluid therapy is indicated in peracute coliform mastitis with cardiovascular compromise, and oxytocin administration combined with frequent stripping of affected quarters accelerates pathogen and endotoxin clearance from the gland cistern.[8]

VACCINATION STRATEGIES: PROGRESS AND PERSISTENT LIMITATIONS

Commercial Vaccine Landscape

Vaccination against bovine mastitis has long been pursued as an attractive alternative to antibiotic-dependent control, yet the quest for a broadly efficacious, commercially viable mastitis vaccine remains unfulfilled despite decades of intensive effort. Commercial vaccines currently available in major dairy markets target three principal pathogen groups: Gram-negative core antigen vaccines (J-5 bacterins, Re-17 vaccines), *S. aureus* vaccines (Lysigin, Startvac, Mastivac), and *S. uberis* surface protein vaccines (Mastitis Shield).[80-82]

J-5 E. coli bacterins, derived from a rough mutant strain expressing the conserved Rd/Re-chemotype LPS core, represent the most extensively evaluated commercial mastitis vaccines. Meta-analyses confirm statistically significant reductions in clinical mastitis severity and case-fatality rates for coliform mastitis, but consistent prevention of IMI occurrence has not been demonstrated.[81,83] Staphylococcal vaccines demonstrate highly variable efficacy across studies, with new infection rate reductions ranging from 0% to 50% and consistently marginal effects on bacteriological cure rates, largely reflecting the immunoevasive capacity of *S. aureus* and the diversity of virulence genotypes in field strains.[82,84,85] Novel Staphylococcal surface-associated protein vaccine candidates have recently demonstrated encouraging results against both *S. aureus* and non-aureus staphylococcal mastitis in dairy cows.[86]

Challenges in Mastitis Vaccine Development

Multiple immunological and biological barriers impede the development of broadly efficacious mastitis vaccines. Antigenic diversity among mastitis pathogens is profound: *S. aureus* alone encompasses more than 2,500 distinct multilocus sequence types, with clonally associated differences in capsular polysaccharide expression (types 5 and 8), biofilm gene presence, and surface protein repertoire rendering strain-specific vaccines clinically inadequate.[28,29] The immune evasion mechanisms of *S. aureus* protein A binding of immunoglobulin Fc regions, CHIPS-mediated neutrophil chemotaxis inhibition, leukocidin-mediated phagocyte killing, and staphylokinase-mediated complement evasion actively counteract vaccine-induced humoral and cellular immunity.[28]

Mammary immunology presents additional barriers unique to this anatomical site. Effective mammary protection requires robust local secretory IgA (sIgA) and IgG2 responses within mammary secretions, yet systemic vaccination primarily induces serum IgG1 antibodies with limited transudation into milk, particularly during mid-lactation when alveolar intercellular junctions are well established.[29] The immunosuppressive periparturient period when vaccine boosters are typically administered to capture peak immune responsiveness before the highest-risk calving window is paradoxically characterized by blunted lymphocyte proliferative responses and reduced antibody production capacity.[47,87]

Next-Generation Vaccine Platforms

Recent advances in vaccinology and biomaterials science offer renewed prospects for mastitis vaccine development. mRNA vaccine technology, dramatically accelerated by COVID-19 pandemic investment, enables rapid antigen design, platform flexibility, and scalable manufacturing without the biosafety constraints of live or killed pathogen production.[88] Veterinary mRNA vaccine candidates targeting *S. aureus* and *Streptococcus uberis* key virulence antigens are currently in preclinical development, with preliminary immunogenicity data demonstrating robust cellular and humoral responses in murine and bovine models.[89,90]

Nanoparticle-based antigen delivery systems including poly(lactic-co-glycolic acid) (PLGA) nanoparticles, lipid nanoparticles (LNPs), and virus-like particles (VLPs) enhance antigen uptake by antigen-presenting cells, provide sustained antigen release, and permit co-delivery of immunostimulatory adjuvants such as toll-like receptor agonists.[91,92] Subunit vaccine candidates utilizing reverse vaccinology approaches mining pathogen genomic sequences for surface-exposed, conserved, immunogenic proteins have identified several promising *S. aureus* antigens, including IsdA, IsdB, ClfA, MntC, and the alpha-toxin H35L mutant.[85,93]

ALTERNATIVE AND COMPLEMENTARY THERAPEUTIC APPROACHES

Bacteriophage Therapy

Lytic bacteriophages viruses that selectively infect and lyse specific bacterial hosts without activity against mammalian cells, eukaryotic microbiota, or unrelated bacterial species have attracted intensifying research interest as precision antimicrobial agents in the context of escalating AMR.[94-96] The theoretical advantages of bacteriophage therapy include extreme host specificity eliminating collateral microbiome disruption, capacity to co-evolve with resistant bacterial populations, self-amplifying dosing kinetics at the infection site, and biofilm-penetrating enzymatic activity of phage-encoded depolymerases.[97,98] Bacteriophage-derived endolysins have also emerged as innovative antimicrobial candidates against bovine mastitis-causing streptococci and staphylococci.[99]

Preclinical evidence from murine mastitis models and bovine quarter challenge experiments supports the bactericidal efficacy of phage cocktails against *S. aureus* and *E. coli* IMI, with significant bacterial count reductions in milk and reduced SCC elevations in phage-treated versus untreated controls.[95,97] A 2024 study evaluating a three-phage cocktail (PHC-1) against *S. aureus* in a lactating mice mastitis model demonstrated significant reductions in bacterial load, alleviated inflammatory responses, and improved mastitis pathology, with efficacy comparable to antibiotic treatment.[60] A subsequent investigation confirmed that a novel bacteriophage cocktail (SW21-SW25) exhibiting activity against 68% of MRSA isolates and 92% of MSSA strains in a BALB/c mastitis model reduced concentrations of IL-1β and TNF-α compared to untreated controls, representing a promising non-antibiotic substitute for managing MRSA bovine mastitis.[35] Furthermore, Cho et al.[100] identified three newly isolated bacteriophages from the Herelleviridae family with stable activity across broad pH (2–12) and temperature (37–70°C) ranges and demonstrated comprehensive genomic characterization of lytic cassettes relevant to *S. aureus* and *S. xylosus* mastitis. However, translation to commercial intramammary therapy faces multiple obstacles: phage stability in the alkaline, lipid-rich milk matrix; rapid phage inactivation by bovine whey proteins and complement; immune-mediated phage neutralization following systemic administration; the narrow host range necessitating pretreatment pathogen identification; and the absence of regulatory approval. Pathways specifically designed for phage veterinary products in most jurisdictions.[101,102] A recent systematic review identified phytotherapy, followed closely by bacteriophage therapy, as the two most extensively researched alternative mastitis treatments, though both face challenges related to compound stability and natural product compositional variability under field conditions.[96]

Antimicrobial Peptides

Host defense peptides (HDPs), including defensins, cathelicidins (LL-37 and its bovine ortholog BMAP-28), lactoferricin, and lactoferrampin, represent components of the mammary gland's innate antimicrobial armamentarium with potent broad-spectrum activity against Gram-positive and Gram-negative mastitis pathogens.[103,104] Unlike conventional antibiotics, HDPs exert antimicrobial effects primarily through membrane disruption mechanisms that are less amenable to target-site resistance development, and many HDPs additionally function as immunomodulators, enhancing neutrophil recruitment, macrophage activation, and resolution of inflammation.[104]

Nisin, a ribosomally synthesized antimicrobial peptide produced by *Lactococcus lactis*, has been extensively evaluated as an intramammary mastitis treatment owing to its potent activity against Gram-positive pathogens including MRSA strains, favorable safety profile, and negligible milk residue concerns.[34,105] Phase II clinical trial data for bovine intramammary nisin formulations demonstrated cure rates comparable to intramammary cloxacillin for streptococcal mastitis.[34,106] Saeed et al.[107] comprehensively reviewed the emerging role of fermentation-derived antimicrobial peptides produced by lactic acid bacteria, demonstrating broad-spectrum activity against major bovine mastitis pathogens including multidrug-resistant isolates, with activity profiles comparable to or exceeding that of nisin. Similarly, Geetha et al.[108] isolated novel antimicrobial peptides from Lactobacillus fermentum (Tp-5) and *Enterococcus* spp. (CRp-2 and CRp-3) fermentation, demonstrating significantly higher inhibitory activity ($P < 0.05$) against *Streptococcus, Staphylococcus*, and *E. coli* isolates than the reference strain Lactococcus lactis. These findings underscore the immense potential of microbially derived peptides as non-antibiotic alternatives for mastitis control in dairy animals.[109]

Probiotics and Mammary Microbiome Modulation

The discovery that the healthy bovine mammary gland harbors a complex resident microbiome including members of the genera Staphylococcus, Streptococcus, Lactobacillus, Lachnospiraceae, and Ruminococcaceae has fundamentally revised the understanding of mammary health and susceptibility to IMI.[110,111] The composition and diversity of the mammary microbiome differ significantly between healthy quarters, subclinically infected quarters, and quarters experiencing clinical mastitis, suggesting that dysbiosis disruption of the normal microbial community may precede or potentiate infection.[111,112]

Intramammary and teat-dip probiotic applications using Lactobacillus and Bifidobacterium strains with demonstrated in vitro anti-staphylococcal and anti-streptococcal activity have been evaluated in a small number of randomized field trials. A controlled trial by Pellegrino et al.[113] demonstrated that post-milking teat dip with a Lactobacillus perolens strain significantly reduced new *S. aureus* and CNS IMI incidence compared to iodine-based teat dip alone. However, probiotic strain selection, optimal application vehicle, dosing regimen, and mechanisms of mammary colonization resistance require substantially more investigation before commercial recommendations can be supported.[112,114]

Immunomodulators and Phytotherapy

Immunomodulatory strategies aimed at enhancing the mammary gland's intrinsic pathogen-clearing capacity without direct antimicrobial activity have attracted growing attention. Recombinant bovine cytokines including interleukin-2 (IL-2), interferon-gamma (IFN-γ), and granulocyte-macrophage colony-stimulating factor (GM-CSF) administered intramammarily in experimental models

enhance neutrophil transmigration, phagocytosis, and oxidative burst activity, accelerating bacterial clearance.[115] Clinical application has been limited by production costs, protein stability in the intramammary environment, and regulatory complexity.

Phytochemical preparations containing bioactive compounds with antimicrobial and anti-inflammatory properties including thymol and carvacrol from oregano and thyme oils, curcumin from Curcuma longa, epigallocatechin gallate (EGCG) from green tea, and eugenol from clove extracts have demonstrated in vitro efficacy against multiple mastitis pathogens and anti-biofilm activity against *S. aureus*.[116,117] In vivo evidence from controlled trials remains limited; standardization of plant material, extraction procedures, active compound concentrations, and safety and residue assessments for intramammary or systemic use in lactating cattle require systematic investigation prior to pursuing regulatory approval pathways.[116] A comprehensive review by Debruyn et al.[96] ranked phytotherapy as the most extensively researched alternative mastitis treatment category, while noting that degradation of active compounds in the milk matrix and variability in natural product composition remain significant barriers to consistent therapeutic outcomes.

GENETIC SELECTION, GENOMICS, AND GENOME EDITING

Genetic Selection for Mastitis Resistance

Genetic selection for mastitis resistance offers a fundamentally distinct and complementary approach to pharmacological and management interventions: rather than treating disease after it occurs or preventing infection through external interventions, it aims to modify the intrinsic susceptibility of cattle populations through selective breeding. The genetic architecture of mastitis resistance is complex, influenced by multiple quantitative trait loci (QTL) of small-to-moderate individual effect, with overall heritability estimates for clinical mastitis and SCC ranging from 0.04–0.10 and 0.10–0.15, respectively, across various populations.[118,119]

Genomic selection, enabled by the availability of high-density SNP arrays and reference population data, has substantially increased the accuracy of estimated breeding values (EBVs) for mastitis-related traits compared to traditional BLUP approaches, particularly in young animals with limited phenotypic records.[120,121] Recent convergence of genomic selection and artificial intelligence is redefining precision breeding in dairy cattle, enabling earlier, more accurate, and multi-trait selection for health, fertility, climate resilience, and economic efficiency, with key applications including improved resistance to mastitis and metabolic diseases.[122] Nordic dairy breeds have incorporated mastitis resistance as a primary breeding goal for decades, and in Norwegian Red cattle, clinical mastitis incidence declined from approximately 40% to less than 10% per 100 cow-years between 1975 and 2015, demonstrating the long-term effectiveness of sustained selection pressure.[123]

CRISPR-Based Genome Editing

CRISPR-Cas9 and related genome editing technologies offer the theoretical possibility of introducing specific, targeted modifications to the bovine genome with unprecedented precision and efficiency, potentially achieving in a single generation what selective breeding requires decades to accomplish.[54] Candidate genetic modifications for enhanced mastitis resistance include the introduction of the Staphylococcus simulans lysostaphin gene under mammary-specific promoter control; polymorphic variants at immune response loci (TLR4, CXCR2, CD14, STAT1, LBP) associated with enhanced pathogen recognition; and correction of deleterious variants reducing immune competence.[124,125]

Regulatory, ethical, and public perception challenges represent the principal barriers to genome-edited cattle commercialization for food production. The FDA's 2022 approval of the PRLR-SLICK thermotolerant cattle genome edit represents a landmark regulatory precedent in the United States, but global trade implications and consumer acceptance remain unresolved.[54,125,126]

PRECISION MEDICINE AND DIAGNOSTIC ADVANCES

Rapid Pathogen Identification

The foundational limitation of empirical antibiotic therapy for bovine mastitis treating a pathogen-diverse syndrome with a one-size-fits-all pharmacological response is increasingly being addressed by rapid, accurate cow-side and near-cow diagnostics. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) enables species-level identification of mastitis pathogens from pure culture within minutes, with accuracy exceeding 98% for common mastitis organisms, and has become the standard of care for mastitis diagnostics in veterinary reference laboratories in high-income countries.[127] Multiplex PCR platforms designed for direct milk sample analysis without culture including the PathoProof Mastitis PCR Assay and Mastit4 enable simultaneous detection of 10–15 common mastitis pathogens with sensitivity and specificity exceeding conventional culture for several important organisms.[128,129] Loop-mediated isothermal amplification (LAMP) assays adapted for cow-side use hold particular promise for pathogen identification in field settings and low-resource production systems.[130]

Automated Monitoring and Artificial Intelligence

Automated milking systems (AMS) and inline milk quality monitoring technologies have transformed mastitis detection capacity in large dairy operations. Inline electrical conductivity sensors achieve sensitivities of 70–85% and specificities of 80–90% for clinical mastitis detection across milking robot platforms, and additional inline sensors measuring milk color, somatic cell count estimates, and LDH activity further enhance detection specificity for subclinical mastitis.[131,132]

Machine learning and AI approaches have demonstrated substantial improvements over threshold-based sensor algorithms for mastitis prediction. Random forest, gradient boosting, and deep learning models integrating multisensor data streams have achieved area under the ROC curve (AUC) values of 0.85–0.96 for clinical mastitis detection in validation datasets.[133-135] A 2024 bibliometric review covering Scopus-indexed papers from 2011 to 2021 confirmed that machine learning and mastitis detection were the most rapidly growing research co-terms in the field, with artificial neural networks as the most cited model type.[136] Wang et al.[137] demonstrated that multilayer artificial neural net (MNET) and

random forest algorithms using variables of milk yield, rumination time, and milk electrical conductivity achieved best performance for predicting naturally occurring clinical mastitis at specific lactation stages across commercial farms. A scoping review of 151 studies on AI and data analytics in dairy farms confirmed that timely mastitis detection consistently ranked among the most important applications, accounting for 13% of all reviewed investigations.[138] Infrared thermography-based deep learning applied to udder thermal images has achieved accuracies of up to 87.6%, sensitivity of 96.3%, and specificity of 84.6% for subclinical mastitis detection, though environmental temperature interference remains a limitation.[139] Federated learning approaches utilizing data from multiple farms without compromising data privacy may enable the development of more generalizable prediction models.[135]

Metabolomics and Biomarker Discovery

Milk metabolomics has emerged as a powerful tool for dissecting the biochemical signatures of mastitis, identifying both diagnostic biomarkers and mechanistic insights into pathogen-host metabolic interactions. NMR spectroscopy and mass spectrometry-based metabolomic analyses consistently identify characteristic alterations in mastitic milk, including elevated N-acetyl-β-D-glucosaminidase (NAGase), lactoferrin, serum amyloid A (SAA), cathelicidin, and haptoglobin levels, alongside decrements in lactose, casein, and total fat content.[140,141] Pathogen-specific metabolic signatures have been identified in several studies, raising the prospect of non-culture diagnostic classification.[142]

INTEGRATED MASTITIS CONTROL PROGRAMS

The Classical Five-Point Plan and Its Evolution

The foundational framework for mastitis control is the Five-Point Plan developed by Neave et al.[143] at the National Institute for Research in Dairying (NIRD), Shinfield, United Kingdom. This plan has been continuously validated, refined, and expanded over more than five decades of field implementation and epidemiological research. The original five components comprised: (1) post-milking teat disinfection using iodophor or chlorhexidine dips, reducing new contagious pathogen infection rates by 50–70%; (2) dry cow antibiotic therapy eliminating subclinical infections and preventing new dry-period acquisitions; (3) prompt identification and treatment of clinical cases minimizing chronic infection establishment; (4) culling of chronically infected cows eliminating contagious pathogen reservoirs; and (5) regular milking machine testing and maintenance preventing mechanical teat-end trauma and cross-contamination.[1,143]

Modern ten-point mastitis control programs extend the original framework to encompass pre-milking teat disinfection (particularly valuable for environmental pathogen control), systematic recording and analysis of mastitis incidence data, regular SCC monitoring through Dairy Herd Improvement Association testing, environmental hygiene optimization (clean and dry bedding, housing design reducing manure contamination of teat ends), biosecurity protocols for prevention of contagious pathogen introduction with purchased cattle, milking management training for farm personnel, and assessment of cow-level risk factors enabling targeted interventions.[1,144,145]

Precision Herd Management

Advanced mastitis control increasingly leverages individual cow-level data integration for precision risk stratification and intervention targeting. Decision support systems incorporating parity, stage of lactation, SCC trajectory, previous mastitis history, bacteriological culture results, genetic merit for mastitis resistance, and body condition score enable identification of high-risk animals for intensified monitoring, early treatment, or selective culling decisions.[146,147] Economic optimization models incorporating cure probability estimates, milk production losses, treatment costs, and replacement value enable data-driven decisions on treating versus culling individual chronic mastitis cases.[3,148]

NUTRITIONAL AND METABOLIC MODULATION OF MASTITIS SUSCEPTIBILITY

Periparturient dairy cows undergo profound metabolic and immunological transitions that substantially increase susceptibility to IMI during the window from approximately three weeks before to three weeks after calving. The convergence of negative energy balance (NEB), hepatic lipidosis, hypocalcemia, and glucocorticoid-mediated immunosuppression creates a period of heightened vulnerability during which 75–80% of all new IMI in a lactation are acquired.[46,47]

Vitamin E and selenium supplementation during the dry period and periparturient period has been consistently associated with reduced mastitis incidence and severity in multiple controlled trials and systematic reviews. Selenium functions as a cofactor for glutathione peroxidase, the primary enzymatic antioxidant system protecting neutrophils from oxidative damage during respiratory burst activity, while vitamin E (alpha-tocopherol) functions as a membrane-bound lipid antioxidant and additionally modulates neutrophil function and lymphocyte proliferative responses. Together, optimizing these micronutrient concentrations in periparturient diets reduces new clinical mastitis incidence by approximately 30–50% compared to deficient control groups.[149,150] Beta-carotene exerts immunostimulatory effects independent of its conversion to retinol, enhancing lymphocyte and neutrophil function.[150] Omega-3 fatty acid supplementation through flaxseed, fish meal, or algal oil modulates the prostaglandin and leukotriene profile of the inflammatory response toward less destructive resolvins and protectins, potentially reducing tissue damage during IMI without impairing pathogen clearance.[151]

FUTURE DIRECTIONS AND EMERGING TECHNOLOGIES

Nanotechnology Applications

Nanotechnology holds considerable promise for advancing both mastitis diagnostics and therapeutics. Nanoparticle-encapsulated antimicrobial formulations including chitosan, PLGA, silver, and milk exosome-based nanovesicle systems offer enhanced intramammary drug delivery with prolonged drug retention, improved biofilm penetration, and reduced systemic absorption compared to conventional formulations.[34,91] A 2024 study demonstrated that milk exosome nanovesicles (mENs) loaded with

aminobenzylpenicillin exhibited 11-fold greater antibacterial activity and fourfold lower MIC values compared to free antibiotic against *S. aureus*, with validated reduction in milk somatic cell counts and bacterial loads in mastitis-affected animals.[152] Castro-Valenzuela et al.[6] comprehensively reviewed the evidence base for nanoparticles as alternative mastitis treatments, confirming their significant relevance across multiple nanoparticle types and delivery systems in veterinary medicine. Nano-biosensors utilizing gold nanoparticle-antibody conjugates, aptamer-functionalized quantum dots, or magnetic nanoparticle immunoassays enable rapid, ultra-sensitive detection of mastitis biomarkers or pathogens in milk at the cow-side without laboratory infrastructure.[92]

Microbiome Engineering

Synthetic biology approaches to mammary microbiome engineering introducing engineered probiotic strains expressing antimicrobial proteins, biofilm-degrading enzymes, or immunostimulatory molecules into the teat canal microbiome represent an emerging frontier in non-antibiotic mastitis prevention.[110,114] The concept of achieving colonization resistance through deliberate shaping of the mammary microbial community represents an analogy to established approaches in human gastrointestinal medicine. Fecal microbiota transplantation (FMT) equivalents for the bovine mammary gland transferring healthy quarter microbiota to dysbiotic or at-risk quarters represent a speculative but conceptually compelling future avenue.[87]

Immunotherapy and Monoclonal Antibodies

Passive immunotherapy using pathogen-specific monoclonal or polyclonal antibodies represents an approach that sidesteps the immune evasion mechanisms limiting active vaccination. Recombinant monoclonal antibodies targeting *S. aureus* virulence factors including anti-alpha-toxin monoclonals (MEDI4893), anti-IsdB antibodies, and anti-CHIPS antibodies have demonstrated efficacy in preclinical bovine mammary infection models and may offer a pathway to adjunctive therapy for refractory *S. aureus* mastitis cases.[93,85]

One Health Integration and Antibiotic Stewardship

The evolution of mastitis control must be situated within the broader One Health framework, recognizing the interconnected health of humans, animals, and ecosystems. Antibiotic stewardship programs (ASPs) for dairy cattle encompassing culture-guided therapy, defined treatment protocols, systematic recording and benchmarking of antimicrobial use, and regular review against resistance monitoring data are increasingly mandated by national legislation, industry quality assurance schemes, and market access requirements.[65,153] The 2019 WHO Critically Important Antimicrobials list designates third- and fourth-generation cephalosporins, fluoroquinolones, and polymyxins as critically important for human medicine, requiring particular justification and restriction for veterinary use.[62,153]

WHY CONTROL, NOT CURE: BIOLOGICAL AND ECONOMIC RATIONALE

The accumulated evidence across more than seven decades of intensive mastitis research compels a clear conclusion: complete eradication of bovine mastitis is biologically implausible, and the scientific and practical focus must remain on controlling the disease to economically and ethically acceptable thresholds. This conclusion rests on several interrelated biological and economic realities.

Environmental pathogen ubiquity represents the most fundamental obstacle to mastitis eradication. *Escherichia coli*, *Klebsiella spp.*, *Streptococcus uberis*, and other environmental organisms exist in astronomical numbers in organic bedding, soil, water, and manure environments that cannot be sterilized in a commercial livestock production setting.[15,16] Even in the highest-biosecurity, best-managed dairy herds in the world, some level of environmental pathogen IMI remains an irreducible biological inevitability given the anatomy of the teat canal and its intermittent environmental exposure during milking.

The mammary gland's inherent anatomical and immunological characteristics create unavoidable vulnerability that management can minimize but not eliminate. The teat canal must open transiently during milking and for variable periods afterward, creating a window of pathogen entry risk. The periparturient immunosuppressive period, driven by the endocrine and metabolic events of parturition, represents a biologically determined vulnerability window that nutritional and management interventions can modulate but not abolish.[46,47]

Economic threshold analysis consistently demonstrates that achieving zero mastitis incidence would require interventions whose costs substantially exceed the economic benefits, making some baseline incidence rate economically rational under any plausible production system.[3,148] The optimal economic mastitis incidence rate defined as the point at which the marginal cost of additional prevention equals the marginal benefit typically falls between 5 and 15 cases per 100 cow-years in well-managed herds.[5]

CONCLUSION

Bovine mastitis exemplifies a paradigmatic 'wicked problem' in animal health science: one characterized by extreme etiological heterogeneity, host-pathogen interaction complexity, environmental unpredictability, and the absence of any single technological solution capable of eliminating the challenge at its root.[1,145] No universal cure has emerged, nor on the basis of current biological understanding can one be anticipated, because mastitis is not a single disease but a syndrome encompassing over 150 distinct microbial causes, each exploiting different virulence strategies and confronting a host whose immune capacity fluctuates dramatically across the production cycle.

Current antibiotic therapy and vaccination approaches provide genuine but modest and pathogen-contingent benefits: adequate for controlling contagious mastitis at the herd level when combined with management measures, but demonstrably insufficient against biofilm-forming *S. aureus*, *Mycoplasma bovis*, environmental streptococci, and emerging AMR strains. Alternatives including bacteriophage therapy,

antimicrobial peptides, probiotics, nanoparticle drug delivery systems, and immunomodulators show compelling preclinical evidence but require substantial translational development before they can be recommended for routine practice. The convergence of pharmacokinetic barriers, biofilm-mediated tolerance, intracellular persistence, and adaptive resistance mechanisms underscores the need for PK/PD-guided, biofilm-aware, and resistance-conscious strategies to support durable next-generation mastitis management.[35]

The most impactful advances in mastitis control over the past decade have emerged not from novel therapeutics alone but from precision diagnostics enabling targeted therapy, automated monitoring systems facilitating early intervention, artificial intelligence-powered risk prediction, and optimized selective dry cow therapy protocols enabling significant antibiotic use reduction without compromising outcomes. These approaches collectively represent the translation of precision medicine principles into practical dairy herd management.

Looking forward to 2026 and beyond, the most promising research directions include mRNA vaccine platforms enabling rapid iteration against evolving pathogen populations, CRISPR-mediated genomic enhancement of mammary immune competence pending regulatory resolution, federated machine learning models enabling real-time population-level mastitis risk prediction, milk exosome and nanoparticle-based intramammary drug delivery systems improving therapeutic concentrations at infection sites while reducing systemic exposure and withdrawal periods, and bacteriophage cocktails tailored for MRSA-predominant herd profiles. Critically, these innovations must be evaluated within integrated One Health and antibiotic stewardship frameworks that ensure their deployment contributes to rather than undermines the long-term sustainability of both dairy production and human antimicrobial therapy.

The defining characteristic of successful mastitis management programs is not the adoption of any single technology but the sustained, evidence-based integration of complementary strategies optimized cow genetics, high-quality transition management, rigorous milking hygiene, targeted diagnostics-guided therapy, selective dry cow protocols, precision monitoring, and continuous performance benchmarking adapted to the specific epidemiological profile and resource environment of each individual herd. Mastitis control, rather than mastitis cure, remains the scientifically defensible, economically rational, and practically achievable goal.[154-158]

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CONFLICT OF INTEREST

The authors declare no conflict of interest in relation to this review.

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