

Bioactive components of human breast milk: Antimicrobial mechanisms, immunomodulatory properties, and laboratory relevance in neonatal care

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ABSTRACT

Background: Human breast milk is a biologically complex fluid whose protective functions extend well beyond nutritional provision. The growing burden of antimicrobial resistance and neonatal vulnerability to infection have renewed interest in characterizing the antimicrobial and immunomodulatory components of human milk through laboratory methods. This narrative review synthesizes current evidence on the major bioactive constituents of human breast milk, with emphasis on mechanisms of action, laboratory quantification and translational relevance for neonatal care.

Methods: A non-systematic literature search was conducted across international databases using terms encompassing lactoferrin, secretory immunoglobulin A, human milk oligosaccharides, antimicrobial peptides, exosomes, milk microbiome, resistome and donor milk processing. Priority was given to studies published in the past 10 years, with earlier foundational references included where appropriate. The substantial heterogeneity in sample collection protocols, measurement techniques and outcome definitions does not lend itself to meta-analytic synthesis; a narrative approach is therefore appropriate.

Results: Human breast milk contains a multi-layered antimicrobial system of proteins (lactoferrin, lysozyme, antimicrobial peptides), secretory IgA, human milk oligosaccharides, extracellular vesicles, bacteriophages, and a resident microbiome. Conventional ELISA fails to resolve glycosylation-dependent functional parameters addressable only by mass spectrometry. HMO composition is 97% *FUT2*-genotype-determined, requiring structural profiling. Holder pasteurization reduces bioactive capacity and generates advanced glycation end products. Gestational age and metabolic status modulate concentrations clinically.

Conclusions: Laboratory characterization of human milk bioactive components represents a clinically relevant and underexplored area of neonatal laboratory medicine. Standardized measurement protocols and evidence-based thresholds are needed to guide clinical decision-making and optimize donor milk banking.

Keywords: antimicrobial resistance, bacteriophage, donor milk, exosomes, human milk, human milk oligosaccharides, lactoferrin, neonatal microbiome, secretory IgA

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INTRODUCTION

The neonatal period represents one of the most immunologically vulnerable phases of human life. The transition from the sterile intrauterine environment to a microbially rich external world occurs in the context of an immature immune system that depends heavily on maternally derived factors for protection. Human breast milk has long been recognized as the optimal nutritional source for neonates, yet its biological complexity extends far beyond macronutrient provision. It is more

accurately described as a living bioactive fluid, capable of modulating immune development, resisting pathogen colonization and shaping the early gut microbiome [1].

The relevance of these properties has acquired new urgency in the context of two interacting global health challenges. Neonatal infections (particularly late-onset sepsis, necrotizing enterocolitis (NEC) and respiratory viral infections) remain leading causes of morbidity and mortality in preterm and term neonates alike, while the global rise of AMR is eroding the effectiveness of antibiotic therapies that have historically underpinned neonatal inten-

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sive care [2, 3]. Against this backdrop, the protective components of breast milk represent not only a natural defense mechanism, but also a potential template for therapeutic innovation. Neonatal sepsis affects an estimated 3 million infants annually worldwide, at an incidence of 2202 per 100 000 live births and a case fatality rate between 11% and 19% [4], figures that underscore the magnitude of the clinical problem and the potential public health impact of optimized use of human milk bioactive constituents.

From the perspective of laboratory medicine, the characterization of human milk bioactive components spans multiple disciplines: clinical biochemistry for the quantification of proteins and oligosaccharides; immunology for the assessment of immunoglobulin isotypes and functional avidity; microbiology for the characterization of milk-resident bacteria and bacteriophages; and molecular biology for the profiling of microRNAs and antimicrobial resistance genes. The integration of these approaches is essential both for understanding individual variation in milk protective capacity and for ensuring the safety and efficacy of donor milk provided to vulnerable neonates.

This narrative review provides a structured overview of the principal bioactive components of human breast milk, by examining their antimicrobial and immunomodulatory mechanisms, the laboratory methods used to characterize them, their behavior under conditions of clinical relevance such as prematurity and gestational diabetes and the laboratory implications of donor milk processing.

The objective of this narrative review is therefore threefold: i) to synthesize current mechanistic evidence on the principal bioactive components of human breast milk, with emphasis on their antimicrobial and immunomodulatory functions; ii) to critically appraise the laboratory methods available for their quantification (from standard immunoassays to mass spectrometry-based proteomics, structural glycomics and metagenomic resistome profiling) by identifying methodological gaps and priorities for standardization; and iii) to assess the translational relevance of these findings for neonatal laboratory medicine, with specific focus on donor milk quality assurance, at-risk clinical populations including preterm neonates and infants of mothers with gestational diabetes and laboratory-based strategies to combat neonatal antimicrobial resistance. Given that only 11.8% of NICUs in technologically advanced health-care systems currently perform any form of human milk analysis beyond standard bacterial culture [5], the need for evidence-based laboratory protocols in this field represents an urgent and largely unmet clinical challenge.

METHODS

This narrative review was conducted following a non-systematic literature search performed between January and March 2025 across the following electronic da-

tabases: PubMed/MEDLINE, Embase, Cochrane Library and Web of Science. The following search terms were used in Boolean combinations: 'human breast milk', 'human milk', 'lactoferrin', 'secretory immunoglobulin A', 'sIgA', 'human milk oligosaccharides', 'HMO', 'antimicrobial peptides', 'exosomes', 'extracellular vesicles', 'microRNA', 'milk microbiome', 'resistome', 'antimicrobial resistance genes', 'bacteriophage', 'donor milk', 'Holder pasteurization', 'HTST pasteurization', 'high-pressure processing', 'multiple reaction monitoring', 'mass spectrometry', 'proteomics', 'glycomics', 'neonatal sepsis', 'necrotizing enterocolitis', 'preterm infant', 'gestational diabetes mellitus', 'neonatal laboratory medicine', 'human milk analysis', 'milk bank'.

The inclusion criteria that we have chosen are the following: peer-reviewed original research articles, systematic reviews, scoping reviews, meta-analyses and narrative reviews which were published in English between January 2015 and February 2025 and which addressed bioactive components of human breast milk with direct relevance to antimicrobial mechanisms, immunomodulatory properties or laboratory quantification in the context of neonatal care. The foundational references published before 2015 were included selectively where they established mechanistic principles which were not superseded by more recent evidence. The exclusion criteria comprise conference abstracts, editorials, non-peer-reviewed sources, studies conducted exclusively in non-human animal models without translational relevance to human neonatal care and articles that are not available in full text. The substantial heterogeneity in sample collection protocols, measurement techniques and outcome definitions across the included literature does not lend itself to meta-analytic synthesis; a narrative approach is therefore appropriate and consistent with established methodology for reviews of this type, as demonstrated by comparable multi-omics scoping reviews in the field [6]. The study selection and data extraction were performed independently by two authors (BS and RI), with some discrepancies which were resolved by consensus. A total of 46 references are included in the final manuscript. Figure 1 presents the literature selection flowchart.

MAIN LITERATURE FINDINGS

Antimicrobial proteins and peptides

Lactoferrin: mechanisms and clinical thresholds

Among the most extensively studied bioactive components of human milk is lactoferrin (Lf), an 80 kDa iron-binding glycoprotein belonging to the transferrin family. Lf exerts antimicrobial effects through multiple independent mechanisms [7]. Iron withholding deprives bacteria of a

critical growth co-factor, and this mechanism is particularly relevant in the intestinal lumen where free iron is limited. The direct bactericidal activity of Lf (mediated through electrostatic interaction between the cationic N-terminal lactoferricin domain and negatively charged bacterial membranes) operates independently of iron saturation

status, thus enabling activity against iron-replete organisms and in conditions of iron supplementation [7]. This mechanistic duality is of direct laboratory relevance: iron-binding capacity assays alone are insufficient to predict Lf antimicrobial activity in vivo; and membrane-disruption assays are necessary to capture the full functional spectrum.

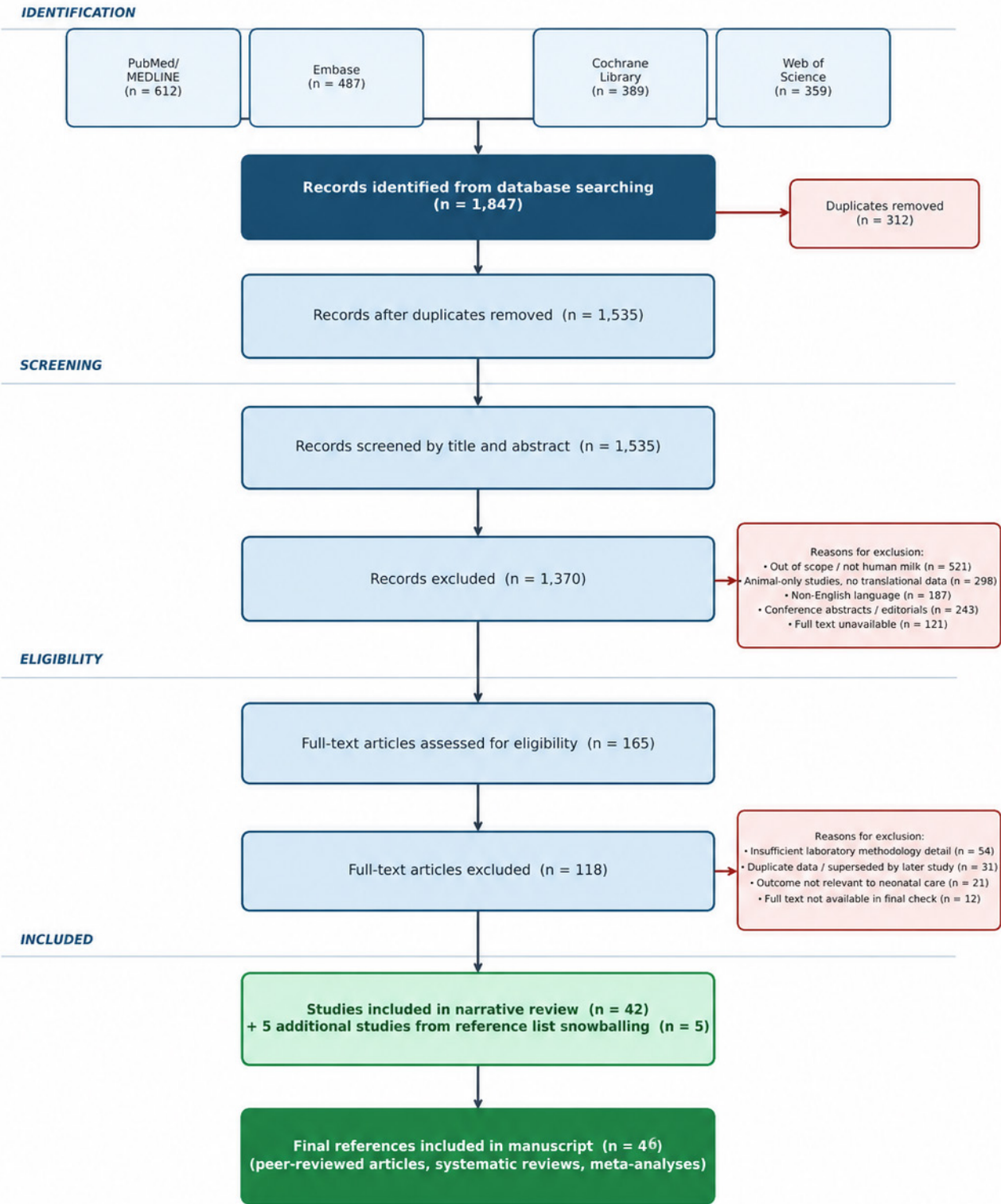


Figure 1. Literature selection flowchart
The records were identified from four electronic databases (PubMed/MEDLINE, Embase, Cochrane Library, and Web of Science) searched between January and March 2025. A total of 46 references were included in the narrative review.

The antimicrobial spectrum of Lf is broad, encompassing Gram-positive and Gram-negative bacteria, fungi and viruses. Of particular clinical importance is its activity against Group B *Streptococcus* (GBS, *Streptococcus agalactiae*), the leading cause of neonatal bacterial sepsis and meningitis. At physiologically relevant concentrations, human milk-derived Lf suppresses GBS growth and disrupts biofilm formation across multiple clinical isolates, with susceptibility patterns varying according to capsular type and sequence type [8]. Colonizing GBS strains are more susceptible than invasive isolates, positioning Lf as particularly effective against the intestinal colonization events that precede systemic infection - a key consideration for laboratories designing functional antimicrobial assays with milk fractions.

Lf concentrations in human milk are highest in colostrum, where they reach 7-8 g/L, and decline to approximately 1-2 g/L in mature milk, although with substantial inter-individual variation [9]. This concentration gradient corresponds precisely to the period of highest neonatal immune vulnerability, therefore suggesting a physiologically adaptive pattern. Laboratory measurement of milk Lf is performed by ELISA or immunoturbidimetry and standardized reference ranges adjusted for lactational stage are needed to support clinical interpretation. Lf concentrations are significantly altered in milk from mothers with gestational diabetes mellitus relative to healthy controls [10] extending the laboratory relevance of Lf quantification beyond research settings to routine clinical monitoring.

The translational potential of Lf as a prophylactic agent in neonatal care is supported by a Cochrane meta-analysis of six randomized controlled trials enrolling over 1000 preterm infants [9]. Enteral Lf supplementation reduces late-onset sepsis (RR 0.59, 95% CI 0.40–0.87) and NEC stage II or III (RR 0.40, 95% CI 0.18–0.86) without adverse effects; in combination with a probiotic, the magnitude of sepsis reduction is substantially greater (RR 0.27, 95% CI 0.12–0.60) [11]. These findings validate the clinical significance of Lf quantification thresholds and position Lf measurement in donor milk quality assessment as an outcome-linked rather than merely descriptive parameter. Questions regarding optimal dose, timing and the equivalence of bovine versus human recombinant Lf remain open and require laboratory-guided resolution.

In our opinion, the mechanistic evidence for LF antimicrobial activity is robust, but the laboratory infrastructure for its functional assessment is inadequate. Critically, glycosylation state is a determinant of LF antimicrobial efficacy: removing fucose from LF glycans enhances *Salmonella typhimurium* binding to epithelial cells; removing sialic acid facilitates *Salmonella enteritidis* adhesion [12]. However, iron-binding capacity assays and ELISA, which constitute the current clinical standard, are blind to glyco-

sylation. MRM mass spectrometry can quantify LF protein and its site-specific glycoforms simultaneously in a single 16-minute UPLC run with RSD < 10% and LOQ of 10-20 fmol [12]. The gap between what is technically achievable and what is clinically implemented represents an organizational rather than a scientific obstacle. The Cochrane meta-analysis supporting LF supplementation [11] is based on low-quality evidence by GRADE criteria; defining optimal supplementation thresholds and LF source equivalence (bovine vs. human) requires precisely the functional characterization that mass spectrometry enables.

Lysozyme and the cooperative antimicrobial system

Lysozyme, a constitutive antimicrobial protein of human milk, cleaves the β -1,4-glycosidic bond between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan layer of bacterial cell walls, with specificity for Gram-positive bacteria. In preterm infants (the population at highest risk of late-onset sepsis and NEC), both Lf and lysozyme concentrations in consumed breast milk are inversely correlated with infection risk [13]. Protein quantification in the milk actually consumed is therefore a more informative quality indicator than batch-level analysis of stored donor milk. The cooperative antimicrobial activity of Lf and lysozyme is substantially attenuated- and in the case of lysozyme, near-completely eliminated- by standard Holder pasteurization, a processing consideration with direct implications for donor milk banking that is discussed in detail below [14].

From our perspective, the near-complete elimination of lysozyme activity by Holder pasteurization [14], combined with the inverse correlation between lysozyme concentrations in consumed milk and infection risk in preterm infants [13], constitutes a compelling argument for lysozyme activity assays (not merely concentration measurement) in donor milk quality panels. Multi-omics analysis of pasteurization effects confirms that HTST preserves lysozyme at >90%, whereas Holder pasteurization results in near-complete inactivation [6]. Despite this evidence, the persistence of Holder pasteurization as the sole standard in most donor milk banks reflects an inertia that laboratory medicine is uniquely positioned to address through outcome-linked quality metrics.

Antimicrobial peptides: the digestive dimension

Beyond these classical proteins, human milk contains a diverse repertoire of antimicrobial peptides (AMPs) generated through the proteolytic digestion of milk proteins. In vitro simulated gastrointestinal digestion of colostrum yields multiple peptide fragments derived from caseins, lactoferrin and α -lactalbumin with significant activity against common neonatal pathogens including *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus agalactiae* [15]. The protective capacity of milk is therefore not simply a property of the intact fluid, but

is actively amplified during digestion, by generating a secondary wave of antimicrobial activity in the intestinal lumen. Conventional antimicrobial assays performed on undigested milk substantially underestimate the total AMP activity experienced by the neonatal intestinal epithelium. Taken together, the evidence on Lf [7], lysozyme [13] and digest-derived AMPs [15] establishes human milk as a multi-layered, context-dependent antimicrobial system whose full characterization requires methodological approaches that account for the physiological transformation of its protein components.

From our point of view, the *in vitro* digestion data establishing that AMP activity is generated and amplified during gastrointestinal processing [15] have a methodological implication rarely acknowledged in the donor milk banking literature: antimicrobial assays performed on undigested milk, which constitute all current quality control procedures, systematically underestimate the total protective activity available to the neonatal intestinal epithelium. Standardized *in vitro* digestion protocols simulating neonatal gastrointestinal physiology, coupled with LC-MS/MS peptide profiling, would provide a more clinically representative assessment of milk antimicrobial capacity. No such standardized protocol currently exists.

Immunoglobulins: Secretory IgA and beyond

The enteromammary pathway and sIgA specificity

Lactating mammary glands are integrated into the secretory immune network via the polymeric immunoglobulin receptor (pIgR) mechanism that governs sIgA export across all mucosal epithelia [16]. Dimeric IgA produced by plasma cells in the mammary lamina propria is transcytosed and released into milk as mature sIgA bearing a covalently linked secretory component - the structural modification that confers resistance to luminal proteolysis and enables functional persistence in the neonatal intestine. On a per-gram tissue basis, the daily IgA output of a single lactating mammary gland approximates that of one meter of intestinal mucosa, quantifying the immunological investment of the maternal secretory immune system in protecting the infant mucosal surface [16].

Human milk is distinguished from serum by the predominance of secretory IgA (sIgA), which constitutes approximately 90% of total milk immunoglobulins. Unlike monomeric IgA, sIgA is a dimeric molecule stabilized by the joining (J) chain and a secretory component (SC) that confers resistance to proteolytic degradation in the intestinal environment - essential for functional protection of the neonatal gut mucosa. sIgA production in the mammary gland is driven by a unique immunological axis: antigen-experienced B cells generated in the mother's gut-associated lymphoid tissue home to the mammary gland via the enteromammary pathway,

resulting in a sIgA repertoire tailored to the mother's enteric pathogen exposure and specifically adapted to the microbial environment the infant will encounter [17]. The functional specificity of milk sIgA cannot be inferred from quantification alone and requires pathogen-specific binding assays to be characterized meaningfully.

In our view, the functional specificity of milk sIgA, tailored to the mother's enteric pathogen exposure via the enteromammary axis, cannot be inferred from total sIgA quantification. Pathogen-specific binding assays are the only approach that meaningfully characterizes protective capacity; however, these are not available in any clinical laboratory setting. Furthermore, standard donor milk banking practice involves pooling milk from multiple donors, which necessarily dilutes individually tailored sIgA repertoires adapted to specific microbial environments. Holder pasteurization reduces total sIgA by 20-30% [14] and multi-omics analysis confirms additional qualitative alterations in the immunoglobulin fraction [6]; HPP and freeze-drying preserve IgA at >80-90% respectively [6]. The argument for alternative processing methods is therefore strengthened both quantitatively and qualitatively by omics-level characterization.

Beyond neutralization: long-term immune programming

The immunoprotective functions of sIgA extend well beyond direct pathogen neutralization. In murine models, maternal milk-derived sIgA protects against allergic asthma by modulating neonatal gut microbiota composition, specifically by limiting early colonization of Th17-inducing bacteria - including *Erysipelatoclostridium ramosum* - in the small intestine [18]. Animals deprived of milk sIgA develop exaggerated mucosal Th17 responses that persist into adulthood and are associated with increased susceptibility to allergic airway disease [18], establishing a mechanistic link between milk immunoglobulins and long-term immunological programming that bridges clinical immunology and pediatric gastroenterology. Anti-Spike sIgA in human milk following maternal SARS-CoV-2 infection retains neutralizing activity for at least one year of lactation post-infection [19], confirming that the immunoprotective contribution of milk sIgA is substantially broader and more durable than traditional models of passive neonatal immunity suggested.

Milk-borne soluble pattern recognition receptors - including soluble Toll-like receptor 2 (sTLR2) - modulate neonatal mucosal responses to microbial antigens independently of immunoglobulins [20]. Milk-borne cytokines such as TGF- β and IL-10, soluble CD14 and epidermal growth factor collectively calibrate the inflammatory tone of the neonatal mucosal epithelium and accelerate barrier maturation [20]. Immunoglobulin profiling of donor milk therefore captures only a subset of the milk's immunomodulatory capacity; measurement of soluble innate immune mediators represents a logical next step for comprehensive donor milk quality characterization.

According to our analysis, the evidence that milk-derived sIgA shapes long-term immunological programming [18, 19] expands the clinical significance of sIgA measurement beyond neonatal passive immunity. However, IgG and IgM profiling (capturing isotypes that mediate allergen tolerance via FcRn-dependent mechanisms and complement-fixing activity, respectively) are absent from virtually all donor milk quality protocols. Multi-omics characterization of pasteurized donor milk reveals that IgG and IgM are partially preserved across processing methods [6], but their functional assessment is not performed. A comprehensive immunoglobulin profiling panel, incorporating isotype-specific quantification and glycoform analysis, represents a logical and technically feasible extension of current donor milk quality assessment.

IgG, IgM and the impact of maternal metabolic status

In addition to sIgA, human milk contains IgG and IgM in lower, but measurable concentrations. The relative proportions of these isotypes shift with gestational age and lactational stage and are substantially affected by maternal health status. Milk from mothers with gestational diabetes exhibits significant differences in sIgA, IgG and IgM concentrations compared to milk from normoglycaemic mothers, with the directional change dependent on lactational stage [10]. Clinical laboratories involved in donor milk banking should therefore incorporate immunoglobulin profiling - not limited to sIgA - into quality assessment protocols, particularly for milk from donors with common metabolic comorbidities.

HMOs: multifunctional bioactive glycans

Structural diversity and prebiotic activity

Human milk oligosaccharides (HMOs) are a structurally diverse family of unconjugated glycans among the most abundant solid components of human milk after fat and protein, yet are not digestible by the infant. Their primary function is biological rather than nutritional: as prebiotic substrates that selectively promote the growth of protective gut bacteria and as decoy ligands that competitively inhibit pathogen adhesion to host epithelial surfaces [21]. More than 200 HMO structures have been identified, with concentrations and compositional profiles varying according to the mother's Lewis blood group and secretor status - individual variation measurable by high-performance liquid chromatography and LC-MS/MS that carries significant clinical implications for the protective capacity of any given mother's milk.

Neutral fucosylated, neutral non-fucosylated and sialylated HMOs exert non-overlapping effects on immune cell populations, with sialylated structures modulating dendritic cell maturation and T cell differentiation in ways that diverge qualitatively from those of fucosylated structures [22]. The individual HMO profile depends on

the expression status of the FUT2 (secretor) and FUT3 (Lewis) genes, which code for distinct fucosyltransferases and generate non-interchangeable glycan classes [22]. This genetic determination of HMO composition, independently of total HMO quantity, establishes a biological rationale for structural profiling rather than for quantitative totals in both research and clinical settings. Laboratories using total HMO measurement by HPLC without structural resolution systematically fail to capture the functional heterogeneity that underlies milk's immunoprotective activity at the individual level.

As we see it, the genetic determination of HMO composition by FUT2 status (with 97% concordance between the rs1047781 SNP and secretor phenotype [23]) establishes that individual HMO profiles are stable, genotype-predictable and fundamentally non-interchangeable between donors. A multi-ethnic cohort study demonstrates that Asian populations exhibit systematically distinct HMO profiles from non-Asian populations, with lower 2'-FL and higher 3-FL, independent of FUT2 genotype frequency [23]; these differences are invisible to total HMO quantification. The laboratories which use HPLC or LC-MS/MS without structural resolution to individual oligosaccharides not only miss functional heterogeneity, but they also apply reference ranges derived from predominantly European and North American cohorts to populations with distinct HMO biology. The population-specific, secretor-status-stratified reference ranges are both necessary and achievable.

Anti-infective activity across pathogen classes

The anti-infective properties of HMOs operate across multiple pathogen classes through mechanistically distinct pathways. Against bacterial pathogens, HMO mixtures from secretor-positive donors exert direct antimicrobial and anti-biofilm activity against GBS, with activity dependent on both HMO structural class and GBS capsular type - convergent molecular defenses against the same neonatal pathogen targeted by Lf [8]. Against viral pathogens, higher infant HMO consumption is inversely associated with RSV infection severity, with mechanistic evidence indicating that HMOs modify host innate responses and reshape the gut microbiome in ways that attenuate systemic viral pathology [24]. In neonatal animal models, HMOs reduce the duration and severity of rotavirus-induced diarrhea through direct competitive inhibition of viral adhesion and modulation of mucosal immunity [25]. Fucosylated and neutral non-fucosylated HMOs (2'FL, 3FL, LNT) increase transepithelial electrical resistance in intestinal epithelial monolayers and protect barrier function against inflammatory insults, while sialylated HMOs (3'SL, 6'SL) promote Th1 and Th17 effector responses while suppressing Th2 cytokine production [26]. This structure-dependent functional segregation -

fucosylated HMOs as barrier architects, sialylated HMOs as immune modulators - has direct implications for the interpretation of HMO profiling data in donor milk quality assessment and clinical research. The measurement of HMO structural classes by LC-MS/MS rather than bulk quantification is therefore a laboratory priority for translating HMO biology into actionable clinical metrics [26].

NEC prevention and clinical evidence

The sialylated HMO disialyllacto-N-tetraose (DSLNT) prevents NEC in neonatal rat models and thus lower DSLNT concentrations in mother's own milk are associated with NEC development in preterm infants [27]. Not all HMOs are therefore equivalent: individual structures map onto distinct clinical outcomes, a central methodological challenge that argues strongly for the adoption of structural profiling rather than total HMO quantification in both research and clinical laboratories. More rigorously controlled clinical trials and better standardized laboratory methods for HMO profiling in clinical milk samples are needed, a gap that remains partially unaddressed [28].

Based on our findings, the DSLNT-NEC relationship [27] is one of the few examples in human milk research where a specific molecular structure maps onto a discrete, high-mortality clinical outcome with a defined mechanistic pathway. The fact that this finding has not generated a standardized clinical assay for DSLNT in donor milk reflects a translation gap that is organizational rather than technical: the analytical capability exists, but the regulatory and institutional framework for incorporating it into quality standards does not. This gap is not acceptable given the 20-30% mortality of NEC in affected preterm infants [29].

HMOs and the neonatal resistome

Bifidobacterium-dominated gut microbiota in breastfed infants - driven in large part by HMO-mediated selective enrichment of Bifidobacterium longum subsp. infantis - is associated with significantly lower load and diversity of antimicrobial resistance genes (ARGs) during the first year of life, independently of antibiotic exposure [30]. This mechanistic link between HMO-driven microbiota composition and AMR gene burden positions HMO profiling of breast milk as a novel laboratory biomarker of neonatal AMR susceptibility with direct implications for population-level AMR surveillance strategies.

Extracellular vesicles, exosomes, and microRNAs

Human breast milk contains a substantial population of extracellular vesicles (EVs), particularly exosomes: membrane-enclosed nanoparticles (30-150 nm) originating from the endosomal pathway. These particles carry a diverse cargo of proteins, lipids and non-coding RNAs - most notably microRNAs (miRNAs) - that survive the acidic and enzymatic conditions of the neonatal gastrointestinal tract and are absorbed by intestinal epi-

thelial cells [31]. Their characterization requires specialized techniques including nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM) and high-throughput proteomic and transcriptomic profiling - methods not yet part of standard laboratory medicine workflows but rapidly becoming more accessible.

Gestational age is a critical determinant of EV bioactivity. Term and preterm transitional human milk EVs share certain functional properties - both reduce IL-1 β secretion and inhibit inflammasome-induced pyroptotic cell death, a mechanism directly relevant to NEC pathogenesis - but diverge in others: only term EVs inhibit IL-6 secretion in epithelial cells and reduce IL-1 β expression in macrophages following inflammatory stimulation [32]. The CD14 surface marker is enriched on preterm EVs relative to term EVs, suggesting differential membrane composition that influences cellular uptake and downstream signaling [27]. The protective EV repertoire of preterm mothers' milk is therefore not equivalent to that of term mothers and gestational-age-stratified EV characterization - already technically feasible by NTA and flow cytometry with surface marker panels - should be incorporated into EV-based quality metrics for donor milk programs serving extremely preterm infants.

Proteomic and miRNA characterization of human breast milk exosomes identifies 367 miRNAs and a rich repertoire of immune-related proteins enriched in KEGG pathways associated with innate immunity, apoptosis regulation and pathogen defense [33]. The most functionally prominent miRNAs target mRNAs encoding inflammatory signaling mediators, suggesting that milk exosomes calibrate neonatal immune activation thresholds and may limit the dysregulated inflammatory cascade that drives intestinal injury in NEC. The exosomal protein cargo includes lactoferrin, lysozyme, butyrophilin and complement pathway components, therefore indicating that exosomes serve as delivery vehicles protecting antimicrobial proteins from luminal degradation and targeting them to mucosal epithelial cells [28]. The exosomal miRNA cargo acts as a molecular bridge between maternal immunity and neonatal mucosal homeostasis, shaping the gut microbiota through mechanisms independent of, but synergistic with, those mediated by HMOs and sIgA [31]. Current laboratory methods for milk exosome isolation and characterization are not yet standardized and significant inter-protocol variability in yield and purity represents a key obstacle to translating these findings into clinical laboratory practice.

In our view, the EV and exosome literature present the most substantial near-term translation challenges of any component reviewed here. Significant inter-protocol variability in EV isolation yield and purity, absence of reference materials and the specialized instrumentation require-

ments of NTA and TEM collectively preclude clinical implementation in the short term. The gestational-age-dependent differences in EV bioactivity [32] (specifically that only term EVs inhibit IL-6 secretion and reduce IL-1 β expression in macrophages, while preterm EVs differ in CD14 surface marker expression) have direct implications for donor milk programs: term donor milk EVs may be functionally mismatched to the specific inflammatory needs of extremely preterm recipients. Multi-omics approaches are necessary to characterize these differences systematically [6].

Milk microbiome and neonatal gut resistome

Composition, origins and colonization function

Human breast milk harbors a diverse bacterial community, which challenges the long-held assumption of milk sterility and opens an entirely new dimension of its biological activity. The milk microbiome is dominated by *Staphylococcus*, *Streptococcus*, and *Lactobacillus* species, with *Bifidobacterium* present particularly in milk from healthy vaginally-delivering mothers. Its composition is influenced by delivery mode, antibiotic use, gestational age and maternal health status [34]. Primary succession of *Bifidobacterium* species in the neonatal gut creates a state of colonization resistance against pathobionts including Enterobacteriaceae and *Staphylococcus aureus*, mediated through competitive exclusion and the production of short-chain fatty acids and bacteriocins [35]. This protective succession depends critically on *B. longum* subsp. *infantis*, a subspecies carrying a unique gene cluster for HMO import and catabolism that has declined markedly in prevalence in industrialized-nation infant microbiota [36]. Probiotic *B. infantis* supplementation significantly shifts the gut microbiome toward a *Bifidobacterium*-dominated profile, with dose and timing of supplementation emerging as critical determinants of efficacy- parameters amenable to laboratory-guided optimization [37].

Resistome dynamics and vertical ARG transfer

The breast milk resistome of healthy lactating women harbors multiple ARG classes carried on mobile genetic elements, with metagenomic evidence of potential transmission to the infant gut [2]. The early-life gut resistome in preterm neonates is shaped by gestational age, mode of delivery and feeding type, with breastfed neonates consistently exhibiting lower ARG diversity than formula-fed counterparts [36]. The *Bifidobacterium* abundance-resistome relationship is independent of antibiotic use, confirming a direct microbiological mechanism rather than a confounding effect of antibiotic selection pressure [30]. Breastfeeding reduces neonatal colonization by AMR organisms across a range of pathogen species through multiple simultaneous mechanisms- direct antimicrobial protein action, microbiome shaping and infection rate reduction [2, 3]. The integration of resistome profiling via targeted PCR or shotgun metagenomics into neonatal surveillance programs is therefore warranted, particularly in settings where AMR is endemic.

We consider that the resistome data [30, 36] reframe donor milk quality assessment in terms that standard bacterial culture cannot address. Metagenomics-based resistome profiling in preterm neonates demonstrates that gestational age, antibiotic exposure, probiotic administration and feeding type independently shape ARG carriage: with *E. coli*, *K. pneumoniae*, and *K. aerogenes* carrying the highest ARG burdens [36]. Culture-based microbiological screening of donor milk detects pathogenic contamination, but it is blind to the ARG burden on mobile genetic elements carried by non-pathogenic organisms. The integration of targeted resistome PCR panels into donor milk banking quality frameworks is technically feasible and should be considered a medium-term priority in high-AMR settings.

Bacteriophages and the human milk virome

The human milk virome has until recently received comparatively little scientific attention. Methodological advances in viral metagenomics have enabled the characterization of milk viromes in clinical cohorts, revealing more than 200 distinct phage species, with considerable inter-individual and intra-individual variation dominated by members of the Caudoviricetes class targeting *Staphylococcus*, *Streptococcus*, and *Enterococcus*- the same genera most abundant in the milk microbiome [38]. This correspondence is consistent with co-evolution of the phageome and microbiome within the mammary gland ecosystem.

Milk bacteriophages exert biological effects through at least two mechanisms: direct modulation of microbiome community composition through predator pressure on specific bacterial populations and direct antimicrobial activity mediated by phage-encoded endolysins. In preterm infants with NEC, milk bacterial composition does not correlate significantly with NEC development per se [39], suggesting that bacteriophage-mediated effects on microbiome architecture are partially uncoupled from total bacterial load. Virome diversity increases with lactational stage and differs significantly between cesarean-delivered and vaginally-delivered dyads [40], consistent with the view that the milk virome, like the microbiome, reflects the mother's microbial history. Standard pasteurization protocols have not been systematically validated against the full diversity of phage species present in donor milk pools, a gap that should be acknowledged in evidence-based quality assurance frameworks.

Donor milk processing: Laboratory considerations for bioactive preservation

Holder pasteurization: efficacy and bioactive costs

When a mother's own milk is unavailable, pasteurized donor human milk (DHM) is recommended by major neonatal societies as the optimal alternative for very low birth weight and preterm infants. The biological benefits of DHM over formula are well established- lower rates of

COMPONENT	CONVENTIONAL METHOD	LIMITATION	ADVANCED METHOD	CLINICAL OUTPUT
Lactoferrin (LF)	ELISA Immunoturbidimetry	Quantifies total LF; glycoforms unresolved; functional avidity unknown	MRM Mass Spectrometry (UPLC-QqQ) Site-specific glycoforms	Glycoform-resolved LF conc. + functional state; donor milk quality index
Secretory IgA (sIgA)	ELISA Nephelometry	Total sIgA only; pathogen specificity not captured	Pathogen-specific binding assays; MRM glycoproteomics	Functional sIgA repertoire; post-pasteurization preservation index
Human Milk Oligosaccharides (HMOs)	Total HMO by HPLC (bulk quantification)	Misses structural class differences; FUT2/FUT3 variation ignored	LC-MS/MS structural profiling (19+ species) HPLC-amide column	FUT2-stratified profiles; DSLINT as NEC biomarker; resistome link
Milk Microbiome & Resistome	Standard bacterial culture (CFU/mL)	Blind to ARGs on mobile elements; no community data	Shotgun metagenomics 16S rRNA sequencing Targeted ARG PCR	AMR susceptibility profile; Bifidobacterium colonization index
Extracellular Vesicles & Exosomes	Not assessed in routine practice	NTA/TEM not standardized; no clinical reference range	Nanoparticle Tracking Analysis (NTA) + TEM Proteomic/c-mRNA profiling	EV concentration & mRNA cargo index; gestational-age stratified

Conventional methods (widely available)

Current methodological limitations

Advanced methods (reference laboratory)

Clinical output / quality metric

Priority for standardization

★ Priority for clinical laboratory standardization: MRM glycoproteomics (LF, sIgA) | LC-MS/MS HMO structural profiling | Metagenomic resistome analysis ★

Figure 2. Analytical workflow for laboratory characterization of human breast milk bioactive components: From conventional methods to functional profiling
Abbreviations: ARG- antimicrobial resistance gene, LC-MS/MS- liquid chromatography tandem mass spectrometry, MRM- multiple reaction monitoring, NTA- nanoparticle tracking analysis.

NEC, late-onset sepsis and feeding intolerance - yet pasteurization significantly alters bioactive composition in ways that are clinically relevant and represent a core laboratory medicine concern. Standard Holder pasteurization (HHP; 62.5°C for 30 minutes) effectively inactivates most pathogenic bacteria and the majority of viral pathogens including CMV and hepatitis A virus [14]. However, HHP reduces Lf activity by approximately 25–50%, eliminates lysozyme activity almost entirely, reduces sIgA concentrations by 20–30% and partially degrades HMOs and bile salt-stimulated lipase [14]. The net effect is a milk product that retains structural macronutrients, but has significantly diminished functional antimicrobial and immunomodulatory capacity. The milk microbiome, a component increasingly understood as a functional contributor to neonatal colonization resistance [35], is eliminated entirely by HHP.

Alternative processing technologies

Alternative technologies have been developed to improve bioactive preservation. High-Temperature Short-Time (HTST) pasteurization (72°C for 15 seconds) achieves equivalent or superior viral inactivation to Holder while preserving a significantly higher proportion of Lf activity, lysozyme, IgA and total protein content, as confirmed by comprehensive proteomic analysis [41]. High-pressure processing (HPP), a non-thermal approach using hydrostatic pressure, shows excellent preservation of CMV neutralization activity and immunological proteins [42] although its effectiveness against all relevant viral pathogens in pooled donor milk has not been comprehensively established. A multicentre randomized controlled trial comparing HTST- versus Holder-pasteurized DHM in extremely low birth weight infants found no significant difference in primary clinical outcomes, although trends toward improved microbiome composition and reduced NEC incidence in the HTST group invite further investigation in adequately powered trials [43]. The absence of significant clinical separation between processing methods despite measurable biochemical differences highlights the importance of laboratory biomarker studies in identifying which specific bioactive components drive clinically meaningful outcomes.

Zinc, MFGM, and emerging quality indicators

Beyond the major protein classes, additional molecular targets have emerged as relevant quality parameters. Zn²⁺ ions present in human milk at physiologically relevant concentrations inhibit contaminating bacteria including *Bacillus cereus* and *Staphylococcus epidermidis* through membrane deformation, supporting free zinc measurement by ICP-MS or colorimetric assay as a novel indicator of donor milk microbiological quality [44]. MFGM-enriched whey fractions display in vitro antiviral activity against RSV, adenovirus and human metapneumovirus [45], identifying MFGM integrity as a relevant quality parameter that

standard processing methods partially disrupt. Laboratory methods to assess MFGM integrity, including phospholipid profiling and proteomic analysis of MFGM-associated proteins, may provide clinically informative metrics beyond standard bacterial culture that could be incorporated into next-generation donor bank quality frameworks.

In our opinion, the multi-omics scoping review of donor milk processing [6] reveals that Holder pasteurization generates Maillard reaction products and advanced glycation end products (molecular modifications not assessed by any current standard quality panel and with potentially detrimental biological effects). The fact that the Garcia-Lara RCT [43] found no significant clinical separation between HTST- and Holder-pasteurized milk does not establish bioequivalence; it establishes that composite clinical endpoints in trials of this size are insufficient instruments for detecting the differential effects of specific bioactive components. By taking into account that HTST preserves IgA and lysozyme at > 90% [6], HPP preserves immunological proteins at > 80% and CMV neutralization capacity comparably to unpasteurized milk [6], and freeze-drying achieves > 90% preservation across most bioactive classes [6], this is a hierarchy of preservation that current clinical practice does not reflect. Only 11.8% of US NICUs perform any form of human milk compositional analysis [5], confirming that the methodological evolution advocated here must begin with basic infrastructure before advanced omics implementation.

Special clinical contexts: prematurity and GDM

Two clinical populations require specific discussion because the bioactive composition of their milk deviates systematically from population averages in ways that have direct laboratory and clinical consequences.

NEC carries a mortality rate between 20% and 30% in affected preterm infants, with highest rates in those requiring surgical intervention; affected survivors face a nearly 25% risk of neurodevelopmental delay [29]. NEC pathogenesis represents a convergence of intestinal immaturity, dysregulated TLR4-mediated innate immune signaling and abnormal microbial colonization [29], each of which is directly modulated by components present in human milk including sIgA, HMOs and the milk microbiome. The laboratory documentation of human milk's NEC-protective bioactive components in individual preterm mothers, and their quantitative changes over the first weeks of lactation, constitutes a clinically actionable research agenda for neonatal laboratory medicine.

Preterm birth before 32 weeks is associated with a distinct immunological context in which both the neonate and the mammary gland operate outside the developmental timeline for which the maternal-infant biological system is designed. Milk from mothers delivering preterm

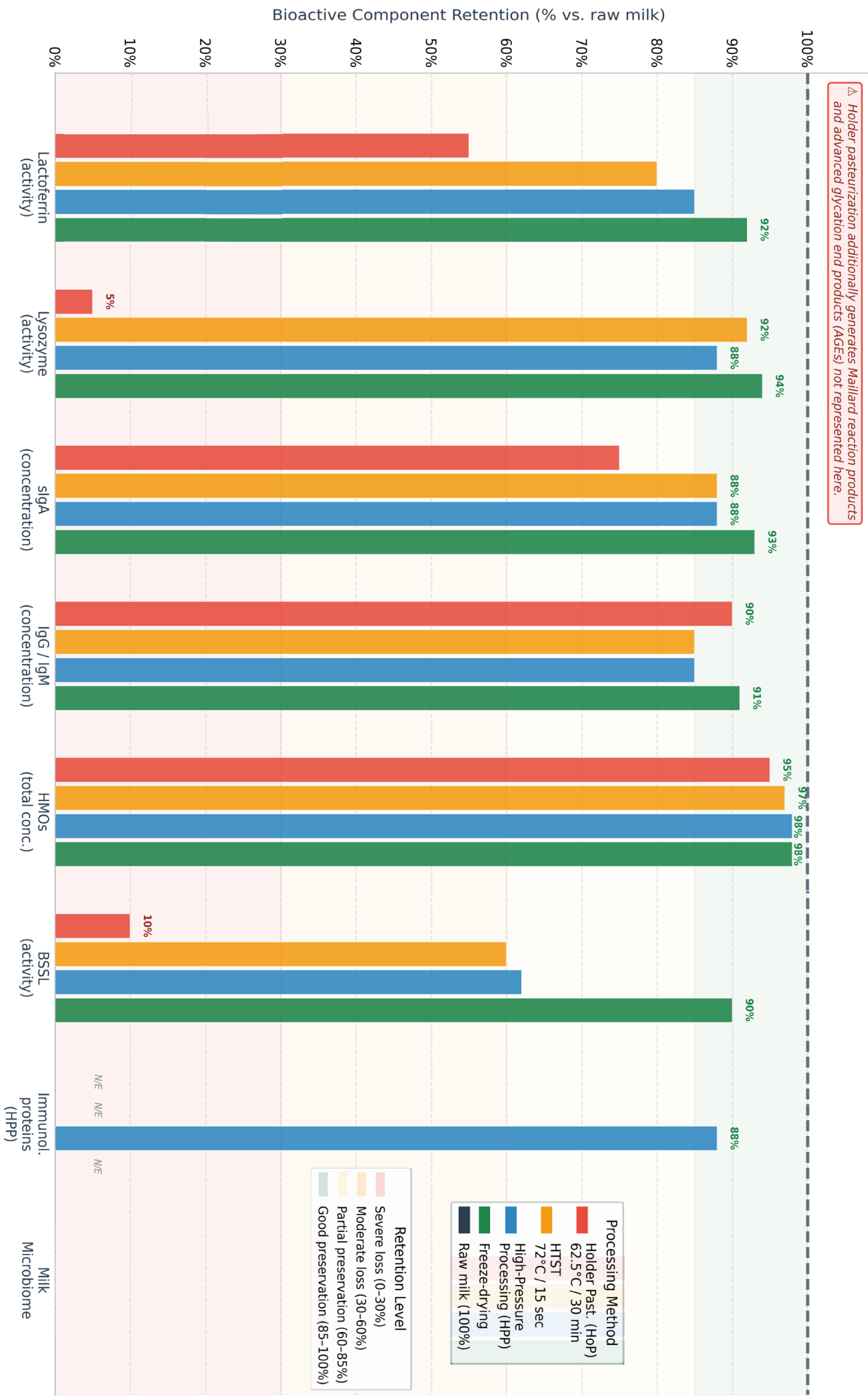


Figure 3. The impact of donor human milk processing techniques on bioactive component retention relative to raw milk
Data derived from multi-omics analyses [6,41,42]. Abbreviations: BSSL - bile salt-stimulated lipase, HOP - Holder pasteurization, HTST - high-temperature short-time, HPP - high-pressure processing, N/E - not evaluated.

Table 1. The main bioactive components of human breast milk: characteristics and practical aspects

Component	Concentration (mature milk)	Laboratory method	Main mechanism(s)	HP effect	Key reference(s)
Lactoferrin	1–2 g/L (colostrum: 7–8 g/L)	ELISA, immunoturbidimetry	Iron sequestration, membrane disruption, biofilm inhibition	Reduced ~25–50%	[7, 14]
Lysozyme	40–160 mg/L	ELISA, turbidimetry	Peptidoglycan cleavage (Gram-positive)	Near-complete loss	[13, 14]
Antimicrobial peptides	Traces (digest-derived)	HPLC-MS/MS post-digestion	Membrane disruption, direct bacteriolysis	Insufficient data	[15]
slgA	0.5–2 g/L (colostrum: up to 10 g/L)	ELISA, nephelometry	Pathogen neutralization, Th17 modulation, microbiota shaping	Reduced ~20–30%	[14, 18]
IgG, IgM	< 0.5 g/L	ELISA	Complement activation, opsonization	Partially preserved	[10]
HMOs	5–15 g/L	HPLC, LC-MS/MS	Prebiotic, competitive pathogen inhibition, resistome reduction	Partially preserved	[21, 30]
Exosomes/EVs	10 ⁹ –10 ¹¹ particles/mL	NTA, TEM, proteomics	miRNA delivery; mucosal immune calibration	Data insufficient	[33]
Microbiome	10 ³ –10 ⁴ CFU/mL	16S rRNA seq., metagenomics	Competitive exclusion; Bifidobacterium seeding	Eliminated	[34, 35]
Bacteriophages	Variable	Viral metagenomics	Microbiome predation; endolysin activity	Partially inactivated	[38]
Free zinc (Zn ²⁺)	0.3–3 mg/L	ICP-MS, colorimetric assay	Membrane disruption; metalloprotein inhibition	Partially preserved	[44]

Abbreviations: ELISA- enzyme-linked immunosorbent assay, EVs- extracellular vesicles, HMOs- human milk oligosaccharides, HP- Holder pasteurization, HPLC-MS/MS- high-performance liquid chromatography coupled with tandem mass spectrometry, ICP-MS- inductively-coupled plasma mass spectrometry, NTA- nanoparticle tracking analysis, slgA- specific IgA, TEM- transmission electron microscopy.

contains significantly higher concentrations of innate immune factors- including Lf, lysozyme and IL-8- compared to term milk, suggesting a physiological adaptation that partially compensates for the greater immune vulnerability of the preterm infant [13]. The reduced HMO complexity and altered microbiome composition of preterm milk may nonetheless limit its Bifidobacterium-enriching capacity and predispose preterm infants to a higher-risk resistome profile [46], a conclusion directly aligned with the gestational-age-stratified resistome data [36]. Pre-term infants receiving their own mothers' milk therefore still face a compounded AMR vulnerability that merits active laboratory monitoring rather than the passive assumption that any breast milk exposure is adequate.

Gestational diabetes mellitus (GDM) affects an estimated 14% of pregnancies globally and is associated with altered inflammatory signaling in the mammary gland. Lf, slgA, IgG and IgM profiles in milk from GDM mothers are significantly altered compared to normoglycaemic controls at multiple time points during lactation, with particularly notable reductions in slgA in colostrum and transitional milk- precisely the period of highest neonatal immune vulnerability [10]. Neonates born to GDM mothers, who already face elevated risks of neonatal hypoglycemia and NICU admission, may therefore receive breast milk with attenuated immunological capacity- a fact that clinical laboratory services are uniquely positioned to document and address through targeted bioactive profiling.

DISCUSSION

The evidence synthesized in this review demonstrates that human breast milk functions as an integrated, context-dependent antimicrobial and immunomodulatory system whose full characterization substantially exceeds the capacity of current routine laboratory workflows. The evidence-based evaluation of the available literature reveals four principal methodological tensions with direct implications for clinical laboratory practice.

First, the analytical gap between concentration and function is most acutely illustrated by the case of lactoferrin. Conventional ELISA and immunoturbidimetry quantify total LF mass without resolving its glycosylation state; however, glycosylation is mechanistically determinant: the removal of fucose increases *Salmonella typhimurium* binding to epithelial cells; and the removal of sialic acid enhances *Salmonella enteritidis* adhesion [12]. Mass spectrometry approaches, specifically Multiple Reaction Monitoring coupled to UPLC, resolve site-specific glycoforms simultaneously with protein quantification in a single experiment [12], which provides a functionally informative measurement that immunoassays cannot replicate. The same principle applies to slgA, whose N-glycans directly mediate pathogen binding and whose glycosylation profile is altered in mothers with gestational diabetes [12]. Laboratories relying exclusively on immunoassay quantification of these proteins are measuring a biologically incomplete parameter, which is a conclusion with direct quality assurance implications for donor milk banking.

Second, HMO profiling illustrates the inadequacy of bulk quantification at the population and individual level simultaneously. The *FUT2* rs1047781 SNP explains 97% of secretor/non-secretor phenotypic variance in multi-ethnic cohorts [23], establishing that HMO composition is predominantly genotype-driven and stable across the first months of lactation. However, Asian populations display systematically lower 2'-FL and higher 3-FL concentrations compared to non-Asian cohorts [23]; these differences are invisible to total HMO measurement and directly relevant to the clinical interpretation of research findings across ethnicities. The structure-function segregation between fucosylated HMOs (barrier integrity) and sialylated HMOs (immune modulation) [26] cannot be captured by any assay that does not resolve individual structural classes. HPLC with 19-oligosaccharide profiling [23] and LC-MS/MS represent the minimum analytical standard for clinically meaningful HMO characterization.

Third, the processing-induced alterations in donor milk bioactivity are more extensive than routine quality assurance currently captures. Holder pasteurization not only reduces IgA and LF by 20-80% and eliminates lysozyme activity, but also generates Maillard reaction products and advanced glycation end products (AGEs): molecular modifications undetectable by current standard donor milk quality panels and with potentially detrimental biological effects [6]. Alternative processing methods (HTST, HPP and freeze-drying) each offer different trade-offs: HTST preserves IgA and lysozyme at > 90%, HPP preserves immunological proteins at > 80% while maintaining CMV neutralization capacity and freeze-drying achieves > 90% preservation across most bioactive classes [6]. The absence of comprehensive clinical outcomes data differentiating these methods reflects the absence of validated laboratory biomarkers capable of translating biochemical preservation into clinical benefit: precisely the translational gap that structured laboratory medicine can address.

Fourth, the resistome dimension of milk quality has no current laboratory equivalent in standard donor milk banking protocols. Metagenomics-based resistome profiling in neonatal cohorts demonstrates that gestational age, antibiotic exposure, probiotic administration and feeding type all independently shape ARG carriage [30, 36] and that these effects are detectable and quantifiable. Breastfed neonates consistently carry lower ARG diversity than formula-fed counterparts, confirming a direct mechanistic link between feeding practice and AMR vulnerability that standard bacterial culture is blind to. Integration of targeted resistome PCR panels into donor milk quality frameworks would transform milk banking from a microbiologically safe, but informationally impoverished practice into one aligned with contemporary AMR surveillance priorities.

Finally, the current state of human milk analysis practice in clinical settings underscores the translation gap: a national survey of US NICUs found that only 11.8% perform any form of human milk analysis, with the majority relying on centrifugation-based fat estimation methods of limited accuracy; and that barriers include cost, personnel and absence of standardized protocols [5]. The methodological evolution advocated in this review is therefore not merely an academic aspiration, but a response to documented clinical infrastructure deficits whose resolution is operationally feasible within the near-term planning horizon of reference laboratories.

LIMITATIONS

Several limitations of this review should be acknowledged. First, as a narrative rather than systematic review, the selection of included studies was not exhaustive and may reflect availability and citation bias; however, the methodological heterogeneity across studies (ranging from standard ELISA to MRM mass spectrometry, shotgun metagenomics and multi-omics platforms) renders meta-analytic synthesis inappropriate at this stage, a conclusion consistent with comparable scoping reviews in the field [6]. Second, conventional immunological methods (ELISA and immunoturbidimetry) that remain the standard in most clinical laboratories fail to capture glycosylation-dependent functional parameters that are mechanistically relevant, but accessible only through mass spectrometry [12]; reference values derived from such methods therefore underspecify the biologically active fraction of key proteins. Third, the clinical relevance of mechanisms mediated by exosomal miRNAs and bacteriophages is primarily supported by in vitro and animal model data, with human translational evidence still in early stages; key findings from 2024-2025 have not yet been independently replicated. Fourth, the majority of available data derive from high-income settings, while HMO profiles vary systematically across ethnic and geographic populations: Asian cohorts exhibiting distinct *FUT2*-dependent compositional patterns compared to European and North American reference populations [12] - limiting direct extrapolation of reference ranges to populations where AMR burden is highest. Fifth, the absence of international consensus on alternative processing technologies means that Holder pasteurization, with its documented bioactive costs including generation of Maillard products and AGEs [6], remains the dominant clinical standard in most donor milk banks. Sixth, resistome profiling via metagenomics, while analytically feasible and clinically informative [36], is not yet implemented in any donor milk banking quality framework, thus representing an organizational rather than technical gap.

CONCLUSIONS

Human breast milk is a biologically complex fluid whose antimicrobial and immunomodulatory functions are mediated by an integrated system. The full characterization of such a complex system requires methods well beyond those currently deployed in most clinical laboratories. Three operationally feasible laboratory priorities emerge from this analysis.

First, the transition from immunoassay-based quantification methods such as ELISA, nephelometry, or immunoturbidimetry, to the more advanced mass spectrometry-based functional characterization (specifically MRM for simultaneous protein quantification and site-specific glycoform profiling [12] and LC-MS/MS or HPLC for HMO structural class resolution [45]) is the single highest-priority analytical upgrade for donor milk quality assessment. Current methods measure the presence of bioactive proteins and oligosaccharides; only mass spectrometry-based approaches measure their functional state.

Second, resistome profiling by targeted PCR panels or shotgun metagenomics should be incorporated into neonatal AMR surveillance frameworks and considered as a novel quality dimension for donor milk banking, particularly in settings where AMR is endemic. The evidence that gestational age, feeding type and probiotic intervention independently modulate the neonatal gut resistome [36] positions human milk as an active determinant of AMR vulnerability- a framing that current quality frameworks do not reflect.

Third, the development of gestational-age-stratified, maternal-metabolic-status-adjusted and ethnicity-informed reference ranges for key bioactive components (lactoferrin glycoforms, specific IgA and individual HMO structural classes) is necessary to support evidence-based clinical interpretation. Given that HMO composition is 97% concordant with the *FUT2* genotype [23], population-specific reference ranges calibrated to secretor status are achievable and clinically necessary.

The priorities mentioned so far are not academic aspirations. They are actionable within the near-term planning horizon of reference laboratories engaged in neonatal care and their resolution has direct consequences for the safety and efficacy of donor milk provided to the most vulnerable patients in the healthcare system. In a clinical landscape, where fewer than one in eight NICUs currently perform any form of human milk compositional analysis [6], the standardization of laboratory protocols for milk bioactive characterization represents both an urgent need and an unrealized opportunity for laboratory medicine.

ABBREVIATIONS

AMR – Antimicrobial resistance
 ARG – Antimicrobial resistance gene
 DHM – Donor human milk
 DSLNT – Disialyllacto-N-tetraose
 EV – Extracellular vesicle
 GBS – Group B Streptococcus
 GDM – Gestational diabetes mellitus
 HHP – Holder pasteurization (Holder method)
 HMO – Human milk oligosaccharide
 HPP – High-pressure processing
 HTST – High-temperature short-time
 ICP-MS – Inductively coupled plasma mass spectrometry
 Lf – Lactoferrin
 MFGM – Milk fat globule membrane
 miRNA – MicroRNA
 NEC – Necrotizing enterocolitis
 NTA – Nanoparticle tracking analysis
 RSV – Respiratory syncytial virus
 SC – Secretory component
 sIgA – Secretory immunoglobulin A
 TEM – Transmission electron microscopy

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AUTHORS' CONTRIBUTION

BS – conceptualization, methodology, writing (original draft), writing (review & editing).
 GB – validation, investigation, writing (original draft).
 DL – writing (original draft), writing (review & editing).
 RI – methodology, data curation, writing (review & editing).
 CD – project administration, supervision.
 All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

None to declare.

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