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REVIEW

Phage diversity in human breast milk: a systematic review

Yanping Guo[1] · Ying Liu[1] · Songzhou Xu[1] · Ruolin Zhang[2] · Zhangbin Yu[3] · Wanxiang He[1]

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Abstract

Breast milk is not sterile. The microbiome in human milk serves as a crucial source of early gut microbes for infants, directly impacting the host's health. This microbiome includes bacteria, viruses, archaea, and fungi. Bacteriophages, as key components of the virome, continually prey on bacterial hosts, thereby influencing the development of early gut microbial communities. Pertinent records from various databases, including EMBASE, Cochrane Library, PubMed, and Web of Science, were comprehensively reviewed against inclusion criteria up to March 24, 2025. A checklist was employed to assess the risk of bias in the selected studies. After screening a total of 635 records, we included 5 studies with 182 women and 251 samples. Seven families of bacteriophages were identified, primarily *Herelleviridae*, *Myoviridae*, *Podoviridae*, *Siphoviridae*, *Caudoviridales*, *Microviridae*, and *Inoviridae*. Their abundance varies at different stages of lactation and can be vertically transmitted through breastfeeding. However, due to the limited number of studies and methodological differences, it is not yet possible to determine which maternal and infant characteristics influence the abundance of these bacteriophages.
Conclusion: Human milk contains abundant bacteriophages that bind to specific bacterial hosts and are transmitted vertically from mother to infant, collectively shaping the infant's gut microbiome. Conducting more longitudinal studies on mother-infant pairs will help better determine the composition of bacteriophages in human milk and their functional impact on infant development.

What is Known:
- *Human milk is a source of diverse microbes, including bacteriophages, that contribute to the establishment of the infant gut microbiome.*
- *Bacteriophages can influence bacterial populations by infecting specific bacterial hosts.*

What is New:
- *Human milk harbors abundant and diverse bacteriophages that are vertically transmitted from mother to infant.*
- *Current evidence underscores the need for longitudinal studies to clarify the role of milk-derived bacteriophages in shaping infant gut microbiota and development.*

Keywords Human breast milk · Bacteriophages · Phages · Diversity

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✉ Zhangbin Yu
yuzhangbin@126.com

✉ Wanxiang He
504619292@qq.com

[1] Department of Pediatrics, Peking University Shenzhen Hospital, No.1120 Lianhua Road, Futian District, Shenzhen, Guangdong, China

[2] Department of Neonatology, Nanshan Maternity & Child Healthcare Hospital, 1 Wanxia Road, Nanshan District, Shenzhen, Guangdong, China

[3] Department of Neonatology, Shenzhen People's Hospital (The Second Clinical Medical College, Jinan University, the First Affiliated Hospital, Southern University of Science and Technology), 1017 Dongmen North Road, Luohu District, Shenzhen, Guangdong, China

Abbreviations

PRISMA	Preferred reporting items for systematic reviews and meta-analysis
NICE	National Institute for Health and Care Excellence

Introduction

Human milk is not only the primary source of nutrition for newborns but also plays a crucial role in establishing the infant's early gut microbiota. The microbiota present in human milk can be directly transmitted to the infant, contributing to the development of intestinal homeostasis and exerting a profound influence on immune system maturation and overall health [1–8]. In recent years, in addition to bacteria, viruses—particularly

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bacteriophages—have attracted growing attention as important yet underexplored components of human milk. As a key component of the gut virome, bacteriophages regulate the succession of the gut microbiota by preying on bacterial hosts, thereby playing an essential role in maintaining microbial homeostasis and promoting host health [9–11]. An increasing number of studies suggest that exogenous bacteriophages may hold therapeutic potential for the prevention and treatment of infections in neonates and children [12–14]. Notably, naturally occurring bacteriophages in human milk may begin influencing the development of the infant gut microbiota from birth, exerting immunomodulatory and antimicrobial effects [15, 16].

The human milk microbiome consists not only of aerobic bacteria (e.g., *Staphylococcus*) and anaerobic bacteria (e.g., *Bifidobacterium*), but also of bacteriophages that regulate the dynamics of these microbial populations [17–19]. Through alternating between lytic and lysogenic life cycles, bacteriophages modulate the structure of microbial communities—either by directly lysing their bacterial hosts or by transferring genes that confer new adaptive traits such as antibiotic resistance or virulence factors. These mechanisms can indirectly affect the infant gut microbiota and its metabolic outputs [20–24].

This review aims to systematically summarize the characteristics and potential functions of endogenous bacteriophages in the human milk of healthy mothers. We also examine the influence of maternal and infant factors on bacteriophage abundance in human milk and explore the possible links between these bacteriophages and child health. By integrating current research findings, we seek to draw attention to the understudied area of the human milk virome and provide a foundation for future investigations.

Methods

This systematic review adhered to the standard criteria PRISMA [25]. This research protocol has been registered in the International Prospective Register of Systematic Reviews (PROSPERO CRD42024538915).

Search strategy

Identification was conducted through PubMed, the Cochrane Library, Embase, and Web of Science, searching articles published up to March 24, 2025, without restriction on country or article type. Keywords and synonyms were derived from human breast milk and bacteriophages. The detailed search strategy is outlined in Supplementary Table S1. Additionally, a comprehensive manual search of the reference lists of all selected articles was conducted to ensure that no relevant studies were inadvertently missed during the initial search process.

Study selection and selection criteria

All identified records were downloaded into EndNote X9, and duplicates were subsequently removed. Two independent researchers screened the studies based on their titles and abstracts, respectively. Subsequently, the studies meeting our criteria underwent full-text screening for further evaluation. Any discrepancies were resolved through discussion with a third researcher until a consensus was reached within the team. Inclusion criteria are as follows: (1) observational studies or experimental reports based on humans; (2) breast milk from healthy mothers; and (3) reports on bacteriophage analysis in human breast milk. Exclusion criteria are as follows: (1) duplicate publications; (2) lack of full text; (3) studies without original data (reviews, comments, editorials, or letters); (4) studies with insufficient methodological or outcome information to extract relevant data on bacteriophage identification, diversity, or abundance; and (5) studies on bacteriophages in pathological lactation (abnormal milk production caused by diseases or conditions such as mastitis, ductal obstruction, or endocrine disorders).

Data extraction

A standardized, pre-tested electronic spreadsheet was used to extract data from the included studies. Information extracted included author names, publication year, country, study purpose, study design, study population, human milk collection time and method, human milk storage method, human milk sample size, preterm/full-term status, experimental analysis methods, bacteriophage classification, and host bacteria. Data extraction was independently performed by four authors (YPG, YL, SZX, and RLZ), with conflicts resolved through discussion in pairs and, if necessary, with the involvement of a third authors.

Quality assessment

The quality of the included studies was assessed using a checklist incorporating relevant questions from both NICE checklist for studies reporting correlations and associations [26] and the checklist proposed by Han et al. [27]. In summary, we considered the clarity of the research question and objectives, the clarity of the methods and results descriptions, and the risk of bias in laboratory and statistical methods. Two independent researchers conducted the assessment, with any disagreements resolved through arbitration by a third researcher. The full version of the quality checklist can be found in the Supplementary Material.

Data synthesis

The results of the included studies were summarized in a narrative synthesis, assessing the abundance and diversity of

bacteriophages in human milk and their associated influencing factors. Due to the small number of included studies (only five) and considerable heterogeneity in study design and methods, meta-analysis could not be performed.

Results

Literature search

A total of 635 records were eventually identified, and 185 duplicate records were deleted. Based on the title and abstract screening, 437 unrelated studies were removed. Of the remaining 13 studies, 7 were not eligible according to our criteria (1 focused on bacteriophages in human milk samples provided by women with mastitis, 1 had insufficient bacteriophage information, 2 only mentioned bacteriophages without performing any analysis, 1 was a study on bacteriophages in cow milk, and 2 analyzed bacteriophages in fecal samples). Additionally, 1 was excluded due to lack of access to the full test. Ultimately, we included 5 studies [28–32]. The flow chart is shown in Fig. 1.

Characteristics of included studies

All of the included studies were published between 2017 and 2023. Five studies were reported in English. The participants were recruited from the USA, the UK, Italy, and Turkey, with no relevant studies from China. The included studies varied considerably in terms of their objectives, but not much in terms of quantitative methods, bioinformatics, and statistical methods. Generally, the aims of these studies could be categorized into one or more of the following: (a) optimizing and applying new methods to detect bacteriophages in human milk, and comparing bacteriophage diversity between human milk and infant feces; (b) analyzing the impact of delivery mode, birth weight, and breastfeeding period on the composition of bacteriophages in human milk; (c) investigating the interaction between bacteriophages and the lipidome in human milk; and (d) exploring the mechanisms of bacteriophage transmission between mother and infant and the potential role of bacteriophages in infant health.

Methodologically, all five studies employed untargeted (shotgun) metagenomics. The number of mothers and

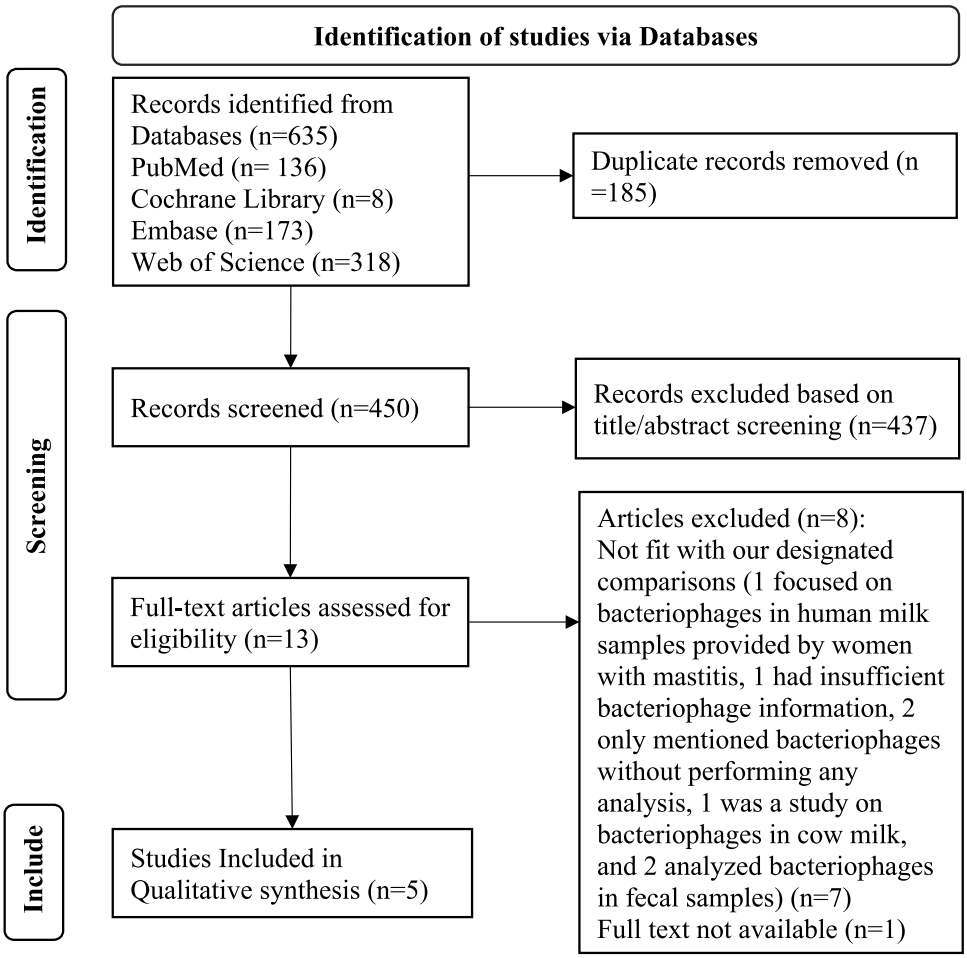

Fig. 1 Flow chart for the selection of studies

samples involved ranged from 4 to 99 participants, with some studies collecting multiple samples per mother, totaling 182 women and 251 samples. Specific characteristics of each study are detailed in Table 1.

Risk of bias of included studies

Overall, there was a sufficient detail regarding research questions, objectives, and participant information (Table 1 and Supplementary Table S2). However, a major limitation across the five studies was the variation in the number of women and samples needed according to each study's objectives, with none reporting sample size calculations. Additionally, details on sample collection and storage were often incomplete. Three studies [28, 31, 32] did not specify how samples were collected (e.g., pump or manual expression).

Descriptions of laboratory analyses were generally adequate, allowing for replication of these methods. Only one study [29] did not report the amount of milk used in the analyses, while the remaining four studies provided details on the milk volume required for phage isolation and DNA extraction. One study [31] confirmed that differences in the initial volume of milk did not affect the relative abundance of phages. All studies reported using different protocols for different sample groups, which were later compared. Specifically, different initial volumes of milk were used in DNA kit evaluations, different kits were used for DNA extraction, and samples came from different cohorts. While all studies analyzed biological replicates, one of the five studies [31] used DNA sequencing technology for replicate analysis.

Data processing and statistical analysis details were largely consistent. Statistical analyses in all five studies were conducted in RStudio. Differences between continuous clinical variables were compared using Fisher's exact test and Kruskal–Wallis rank-sum test, with adjustments made for multiple comparisons [31, 32]. Count data and categorical data were compared using the Mann–Whitney test [29–31].

Bacteriophages and their host types in human milk

Bacteriophages found in human milk are predominantly double-stranded DNA (dsDNA) viruses. Across the five studies, a total of seven families of bacteriophages were identified: *Herelleviridae*, *Myoviridae*, *Podoviridae*, *Siphoviridae*, *Caudoviridales*, *Microviridae*, and *Inoviridae* (see Fig. 2). *Siphoviridae* was reported in all five studies, while *Myoviridae* and *Podoviridae* were reported in four studies each, with the remainder reported once. Yew et al. [32] identified three types of bacteriophages in human milk, with *Siphoviruses* being the predominant dsDNA viruses, comprising up to 80% of the bacteriophage population, followed by *Podoviruses* and *Myoviruses*. Bacteriophages were abundant and prevalent in human milk from the first week of lactation through the first 100 days, with the richness of bacteriophage hosts in preterm human milk gradually increasing. Dinleyici et al. [30] found that bacteriophages dominated both transitional and mature milk samples. However, *Podoviridae* and *Myoviridae* were more abundant in transitional milk, while in mature milk, *Podoviridae* abundance decreased, and *Siphoviridae* became the most abundant. Pannaraj et al. [29] discovered that *Myoviridae* were predominant in the milk of healthy mother-infant pairs, followed by *Siphoviridae* and *Podoviridae*. Duranti et al. [28] identified the *B. longum* phage 10029 in human milk and provided evidence of its vertical transmission.

The taxonomic specificity of phage hosts was inferred based on nucleotide similarity with reference sequences. During sampling, bacteriophages primarily infected genera including *Staphylococcus*, *Streptococcus*, *Propionibacterium*, *Escherichia/Shigella*, *Pseudomonas*, *Enterococcus*, *Clostridium*, and *Bifidobacterium*.

Phage stability

One study [31] evaluated the impact of pasteurization on bacteriophages in human milk. The results showed that bacteriophage nucleic acids persisted in the milk after pasteurization, and there were no significant differences in the concentration, richness, or Shannon diversity of bacteriophages extracted from different volumes (0.1, 0.2, 0.4, and 0.6 mL) of milk. Additionally, the study found shared bacteriophages between newborns' milk and fecal samples, further confirming the vertical transmission of bacteriophages from mother to infant. Additionally, the collection and storage temperatures of human milk samples varied across the five studies [28–32] (as shown in Table 1). Different collection and storage methods may affect the activity and diversity of bacteriophages, potentially impacting their stability.

Factors affecting phage-related aspects

Only two studies [30, 32] have investigated the effects of maternal and infant characteristics on bacteriophages in human milk. Dinleyici et al. [30] first demonstrated the influence of delivery mode, prematurity, gestational age, birth weight, and lactation period (transient or mature milk) on the composition of bacteriophages in human milk. The abundance and diversity of bacteriophages in human milk showed variations based on lactation period (transient or mature milk), gestational age, birth weight,

Table 1 Characteristics of studies included in the systematic review

Author (year)	Study aims	Milk volume to DNA isolation	Number of women/ samples	Preterm/ term	Lactation time (d)	Milk sample collection	Storage temperature for milk sample	Milk fraction	Phage DNA isolation method	Phage DNA profiling method	Phage analyzed	Host analyzed	Open date
Yew (2023) [32]	To characterize the dsDNA phage community composition and lipidome of preterm HM across different lactational ages	200 µL	99/99	Preterm	3–100	NR	− 80 ℃	separation of milk fat and skim milk	Norgen Phage DNA Isolation Kit	Shotgun metagenomics	*Siphoviruses*, *Podovirus*, *Myoviruses*	*Staphylococcus*, *Propionibacterium*, *Enterobacteria/Klebsiella*, *Escherichia*, *Pseudomonas*, and *Enterococcus*	PRJEB58774
Young (2022) [31]	To develop a robust method for isolation of bacteriophages from the smallest possible volumes of BM from mothers to very preterm infants	0.1, 0.2, 0.4, and 0.6 mL	4/4	Preterm	7–22	NR	− 80 ℃	Skim milk and pellet microbial cells	QIAamp MinElute Virus Spin Kit	shotgun metagenomics	*Herelleviridae*, *Myoviridae*, *Podoviridae*, *Siphoviridae*	*Staphylococcus*, *Escherichia*, *Enterobacteria*, and *Klebsiella*	PRJEB49989

Table 1 (continued)

Author (year)	Study aims	Milk volume to DNA isolation	Number of women/ samples	Preterm/ term	Lactation time (d)	Milk sample collection	Storage temperature for milk sample	Milk fraction	Phage DNA isolation method	Phage DNA profiling method	Phage analyzed	Host analyzed	Open date
Dinleyici (2021) [30]	To analyze the impact of delivery mode, birth weight, and lactation period on the composition of maternal breast milk bacteriophages	1.5 mL	44/88	Preterm and term	7–15, 45–90	Hand expression	− 20 ℃	Fat and the supernatant component of the milk were carefully removed	QuickGene filter cassette	Metagenomic	*Podoviridae*, *Myoviridae*, *Siphoviridae*	*Staphylococcus*, *Streptococcus*, *Acinetobacter*, *Enterococcus*, *Clostridium*	PRJEB26810,ERS2488898, ERS24888985)

Table 1 (continued)

Author (year)	Study aims	Milk volume to DNA isolation	Number of women/ samples	Preterm/ term	Lactation time (d)	Milk sample collection	Storage temperature for milk sample	Milk fraction	Phage DNA isolation method	Phage DNA profiling method	Phage analyzed	Host analyzed	Open date
Pannaraj (2018) [29]	To explore the vertical transmission of Bifidobacterium and Bifidobacterium phages between mothers and infants, and study the influence of Bifidobacterium phages in breast milk on the infant gut microbiota	NR	10/10	Preterm and Term	4–10	Manual expression or electric breast pumps	− 80 ℃	Remove cellular and other debris	Qiagen QIAamp DNA MINI kit	Shotgun metagenomics	*Myoviridae*, *Siphoviridae*, *Podoviridae*, *Caudoviridales*, *Microviridae*, *Inoviridae*, DsDNA viruses	*Streptococcus*, *Veillonella*	SRP127558

Table 1 (continued)

Author (year)	Study aims	Milk volume to DNA isolation	Number of women/ samples	Preterm/ term	Lactation time (d)	Milk sample collection	Storage temperature for milk sample	Milk fraction	Phage DNA isolation method	Phage DNA profiling method	Phage analyzed	Host analyzed	Open date
Duranti (2017) [28]	To explore the vertical transmission of Bifidobacterium and Bifidobacterium phages between mothers and infants	1 mL	25/50	Term	7,30	NR	− 20 ℃	whole milk	Illumina Nextera-XT DNA kit	Shotgun metagenomics	*Bifidophages:B. longum phage 10,029*, *B. longum phage 10,035*, *Siphoviridae*	*Bifidobacteria*	SRP102273

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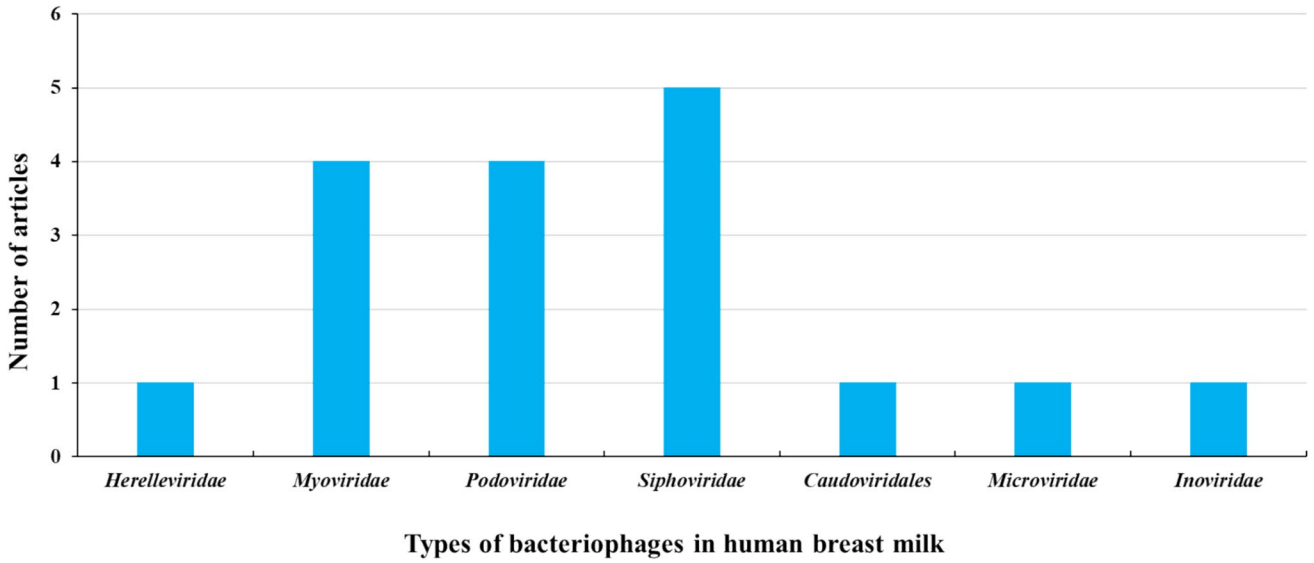

Fig. 2 Taxonomic classification of bacteriophages identified in the systematic review

and delivery mode. Overall, bacteriophages are more abundant in transient human milk than in mature human milk. In transient human milk, *Podoviridae* is more prevalent, while in mature human milk, the proportion of *Siphoviridae* increases, and that of *Podoviridae* decreases. In the large for gestational age group, bacteriophages account for 68.3% in transient human milk and increase to 80.6% in mature human milk. In contrast, the small for gestational age group has lower proportions of bacteriophages in both transient and mature human milk, approximately 45%. In the premature group, bacteriophages constitute 92.1% in transient human milk, with a decrease to 56% in mature human milk. *Myoviridae* is the predominant bacteriophage family in the premature group. In the normal spontaneous vaginal delivery group, *Podoviridae* is the most abundant bacteriophage family in transient human milk, while *Siphoviridae* dominates in mature human milk. Conversely, in the cesarean section group, *Myoviridae* predominates in both transient and mature human milk. Another study by Yew et al. [32] [0] discovered that gestational age and mode of delivery had no significant impact on the abundance and diversity of bacteriophages in preterm human milk. However, a stronger relationship was observed between lipids and bacteriophages in human milk. The higher lipid concentration in preterm milk may facilitate the transfer of bacteriophages to the infant's gastrointestinal tract. As lactation progresses, the proportion of long-chain fatty acids (LCFAs) and polyunsaturated fatty acids increases, which is positively correlated with the relative abundance of double-stranded DNA bacteriophages, such as *Siphoviruses* and *Podoviruses*, known to infect *Staphylococcus* and *Propionibacterium*.

Bacteriophages and infant health

Three studies [28, 29, 32] have analyzed the impact of bacteriophages on infant health. Duranti et al. [28] examined the presence of *Bifidobacterium* species in the milk and infant fecal samples of 25 mother-infant pairs at 7 days and 1 month postpartum. The study confirmed that bacteriophages in human milk can be transmitted to infants through milk, helping to establish their gut microbiota and thereby positively impacting the gut health of infant. Pannaraj et al. [29] analyzed the DNA viruses in human milk and infant fecal samples from 10 healthy mother-infant pairs. The results showed that bacteriophages predominated in both human milk and infant feces, indicating that the extensive transmission of bacteriophages between mother and infant may contribute to the formation and development of the infant gut microbiota, playing an important role in infant health. Yew et al. [32] found that, especially in preterm infants, bacteriophages present in human milk help reduce the incidence of necrotizing enterocolitis (NEC) and late-onset sepsis (LOS), thereby providing a protective effect on the health of preterm infants.

Discussion

This systematic review identified the presence of diverse endogenous bacteriophages in the human milk of healthy mothers. However, due to the limited number of published studies on this topic, only five peer-reviewed scientific studies were ultimately included. These studies had diverse objectives and showed minimal overlap. As research methodologies become more consistent and study characteristics evolve, this

field is rapidly advancing. However, heterogeneity in sample collection and processing within and between studies, along with a lack of standardization, presents significant challenges in human milk research [33, 34]. Future studies should aim to improve transparency and consistency in sampling procedures.

The five studies included in this systematic review demonstrate the presence of various bacteriophages in human milk, with *Siphoviridae* being the most frequently reported, followed by *Myoviridae* and *Podoviridae*. This finding aligns with the prominent bacteriophages identified in current research [35, 36]. Maqsood et al. [36] reported a dominance of these bacteriophages in the human milk virome of HIV-infected mothers. *Siphoviridae* are characterized by long, non-contractile tails with specific terminal adhesion structures that recognize host surface proteins or polysaccharide receptors. Research has focused on bacteriophages infecting lactic acid bacteria (e.g., *Streptococcus* bacteriophages c2 and bIL67), *Escherichia coli* bacteriophages (e.g., λ, HK97), and *Bacillus subtilis* bacteriophage SPP1 [37] [69]. *Myoviridae* have contractile tails with complex attachment structures, including T4 (infecting *E. coli*), PA01 (infecting *Pseudomonas aeruginosa*), and φC31 (infecting *Streptomyces*) [38]. *Podoviridae* possessed short tails with specialized recognition apparatus, such as P22 (*Salmonella*), phiKMV (*P. aeruginosa*), and T7/T3 (*E. coli*) [39].

Bacteriophage nucleic acids remain detectable in human milk even after pasteurization, indicating a degree of thermal stability. Samples as small as 0.1 mL were sufficient for bacteriophage detection in milk from extremely preterm infants. Stinson et al. [40] found that ultraviolet-C irradiation effectively inactivates both thermotolerant and thermosensitive bacteriophages in human milk and water, whereas pasteurization only inactivated the latter (e.g., *Staphylococcus aureus* bacteriophage BYJ20), leaving heat-resistant bacteriophages like *E. coli* bacteriophage T4 unaffected. However, these findings were based on exogenous bacteriophages; effects on endogenous bacteriophages require further validation.

Multiple maternal and fetal factors may influence the diversity and abundance of bacteriophages in human milk and the infant gut, including geographic location, maternal diet, psychological state, mode of delivery, gestational age, antibiotic use, and stage of lactation [6, 8, 30, 32, 41–44]. These factors collectively shape the infant gut microbiota via bacteriophages transmitted through breastfeeding. Given the immaturity of the infant immune system, lytic bacteriophages may suppress pathogenic bacteria and help modulate gut microbial stability. Studies have noted inverse correlations between certain *Lactobacillus* species and their corresponding bacteriophages, potentially reflecting microbiome instability in early life [45, 46]. While bacteriophage abundance is generally higher in infants, whether lytic bacteriophages are more dominant in infant guts compared to adults remains uncertain [47].

This review's main strength lies in its systematic identification of relevant studies and comprehensive analysis of bacteriophage species in human milk, their stability, functions, and influencing maternal and infant factors. We also evaluated study quality and biases, highlighting methodological limitations and providing recommendations for improvement in this emerging field.

It is worth noting that this review used the traditional morphological taxonomy of bacteriophages (e.g., *Myoviridae*, *Siphoviridae*, *Podoviridae*), which does not fully reflect their evolutionary or genetic relationships. In 2021, the International Committee on Taxonomy of Viruses (ICTV) abolished these polyphyletic families, reclassifying them under higher-order taxa such as *Caudoviricetes*, including families like *Kyanoviridae* [48]. Future research should incorporate updated taxonomic systems to enhance ecological and functional insights.

This review confirms the presence of abundant bacteriophages in human milk, which may play important roles in shaping infant gut microbiota and immunity. Future research should prioritize longitudinal studies involving mother-infant pairs to explore temporal dynamics of the human milk and infant viromes, investigate the effects of maternal health, geography, and lactation stages, and elucidate mechanisms by which bacteriophages regulate gut microbiota and immune development. Additionally, greater attention should be paid to how milk processing (e.g., pasteurization) impacts bacteriophage viability, with the ultimate goal of optimizing feeding strategies and exploring therapeutic applications of targeted bacteriophage interventions in neonates.

Conclusion

Human milk contains abundant bacteriophages that can bind to specific bacterial hosts and are vertically transmitted from mother to infant, collectively shaping the infant's gut microbiome. Conducting more longitudinal studies on mother-infant pairs will help better determine the composition of bacteriophages in human milk and their functional impact on infant development.

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Authors' contributions Y.G. designed this systematic review. Y.L., S.X., Z.Y. and W.H. searched aimed articles. Y.G., Y.L., S.X. and R.Z. extracted data. Y.G. wrote the manuscript and submitted the

manuscript. All authors have reviewed and revised the manuscript, and approved the final manuscript as submitted.

Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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