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Systematic Review of the Human Milk Microbiota

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Abstract

Human milk–associated microbes are among the first to colonize the infant gut and may help to shape both short- and long-term infant health outcomes. We performed a systematic review to characterize the microbiota of human milk. Relevant primary studies were identified through a comprehensive search of PubMed (January 1, 1964, to June 31, 2015). Included studies were conducted among healthy mothers, were written in English, identified bacteria in human milk, used culture-independent methods, and reported primary results at the genus level. Twelve studies satisfied inclusion criteria. All varied in geographic location and human milk collection/storage/analytic methods. *Streptococcus* was identified in human milk samples in 11 studies (91.6%) and *Staphylococcus* in 10 (83.3%); both were predominant genera in 6 (50%). Eight of the 12 studies used conventional ribosomal RNA (rRNA) polymerase chain reaction (PCR), of which 7 (87.5%) identified *Streptococcus* and 6 (80%) identified *Staphylococcus* as present. Of these 8 studies, 2 (25%) identified *Streptococcus* and *Staphylococcus* as predominant genera. Four of the 12 studies used next-generation sequencing (NGS), all of which identified *Streptococcus* and *Staphylococcus* as present and predominant genera. Relative to conventional rRNA PCR, NGS is a more sensitive method to identify/quantify bacterial genera in human milk, suggesting the predominance of *Streptococcus* and *Staphylococcus* may be underestimated in studies using older methods. These genera, *Streptococcus* and *Staphylococcus*, may be universally predominant in human milk, regardless of differences in geographic location or analytic methods. Primary studies designed to evaluate the effect of these 2 genera on short- and long-term infant outcomes are warranted. (*Nutr Clin Pract*.2017;32:354-364)

Keywords

breast milk; human milk; microbiota; microbiome; human microbiome; metagenome

Breastfed infants have a decreased risk of developing respiratory tract infections, atopic dermatitis, asthma, obesity, type 1 and 2 diabetes, necrotizing enterocolitis, gastroenteritis, and sudden infant death syndrome.[1,2] Human milk is nutrient rich,[1] and bacterial communities have been identified in human milk by both culture-dependent and culture-independent analyses.[3,4] Human milk has the potential to modulate colonization and development of the immature newborn gut[5] through the transmission of milk-based bacteria.[6,7] As a result, the bacterial content of human milk may directly affect short- and long-term infant health outcomes.[8]

The mission of the Human Microbiome Project is to characterize the human microbiome from multiple body sites, but the investigators did not include human milk as one of the 18 anatomical regions or body sites of interest.[9] Nonetheless, independent studies have analyzed the human milk microbiota.[3,6,7,10–26] Early studies using cultured breast milk isolated only a limited number of genera.[6] Subsequent development of culture-independent methods has allowed for a more complete understanding of the composition and diversity of microbiota present in human milk.* Using ribosomal RNA (rRNA) polymerase chain reaction (PCR), Hunt et al[13] identified a "core" milk microbiota consisting of 9 bacterial genera in milk collected from 16 mothers, while Jiménez et al[24] identified an alternate core microbiota consisting of 7 genera in samples collected from 10 mothers. Only *Staphylococcus*, *Streptococcus*, and *Propionibacterium* were similarly reported in both studies as predominant genera in maternal milk.[13,24] This may be the result of dietary, genetic, or environmental differences affecting the human milk microbiota.[3,13]

This systematic review sought to characterize the diversity and commonalities of the human milk microbiota by

*References 6, 7, 10, 13–15, 17, 20, 24, 26,27.

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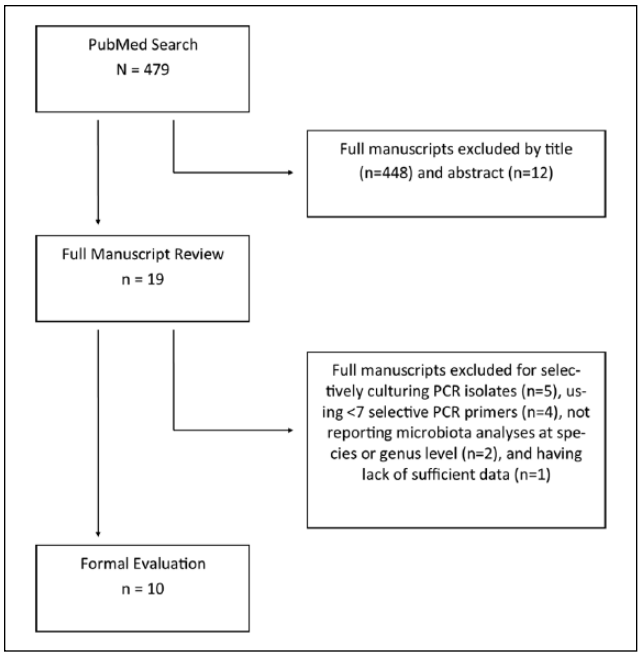

Figure 1. Literature search flow diagram. PCR, polymerase chain reaction.

synthesizing data derived from culture-independent methods using samples obtained from various geographic locations.

Methods

We identified all primary studies indexed in PubMed from January 1, 1964, through June 30, 2015, that described the diversity of healthy mothers' milk microbiota. Search terms included the following: microbiome OR microbiota OR anaerobic OR anaerobe OR metagenome AND (human milk OR breast milk).

We identified eligible studies for this systematic review using the following inclusion criteria: (1) primary study, (2) written in English, (3) exclusive use of human subjects, (4) investigated bacterial diversity and composition of human milk, (5) used culture-independent methods, and (6) reporting primary results at the genus level. The following exclusion criteria eliminated studies from the review: (1) exclusive assessment of the microbiota of colostrum and (2) incomplete/insufficient data set reported.

Two authors independently reviewed PubMed search results. Reviewers assessed each title, evaluated abstracts as necessary, and considered the study for full review. Any disagreements in either the title/abstract or the full manuscript review phases were resolved by consensus. All eligible studies were formally evaluated and included in this systematic review.

Results

The PubMed search yielded 479 articles (Figure 1). We excluded 448 articles by title and 12 by abstract review; 19 articles qualified for full manuscript review. Of those 19, we excluded 5 that did not evaluate the bacterial diversity of human milk, 1 for nonindependent evaluation of the human milk microbiota, and 1 for having an incomplete data set; 12 articles qualified for formal evaluation.

Characteristics of formally evaluated studies are presented in Table 1. Studies were conducted in Spain, Finland, Turkey, Canada, Switzerland, and the United States. Six studies evaluated healthy mothers exclusively, while the remaining 6 compared healthy mothers with mothers who were overweight, were undergoing chemotherapy, and had celiac disease, mastitis, or breast milk jaundice. The definition of maternal health status varied among studies; 6 studies cited the absence of medical conditions,[10,12,21,22,24,26] 1 used maternal self-report,[13] and 5 did not specify how maternal health was defined.[4,6,17,19,20] Aseptic milk collection techniques differed with respect to use of chlorhexidine[21]; a combination of soap, sterile water, and chlorhexidine[6,12,24]; iodine[13,22,26]; aseptic soap[28]; sterile swabs[17]; sterile gloves[19]; sterile collection bag[20]; or undefined.[16] In addition, studies varied with respect to microbial detection methods. Eight studies[6,12,16,17,19,21,22,26] (67%) used conventional rRNA PCR, whereas 4 studies[10,13,20,24] (33%) used next-generation sequencing (NGS), which included 454-pyrosequencing using an Illumina (Illumina Inc, San Diego, CA) platform. As well, the collection timeframe of the included milk samples varied from 4 days postpartum to 6 months postpartum,[4,12,19–22,26] while 5 studies did not identify the postpartum day milk samples were collected.[6,13,17,24,28]

Microbial Presence

Microbial presence was defined by included studies as the genera or species identified in the human milk samples. The genera *Streptococcus* and *Lactobacillus* were identified in 11 studies (91.6%), *Staphylococcus* in 10 studies (83.3%), *Bifidobacterium* in 9 studies (75%), *Enterococcus* in 8 studies (66.7%), and *Propionibacterium* in 6 studies (50%) (Table 2). Differences in methodology resulted in inconsistent detection of bacterial diversity of human milk. Using conventional rRNA PCR, *Lactobacillus* was identified in all 8 studies (100%), *Bifidobacterium* and *Streptococcus* in 7 studies (87.5%), *Staphylococcus* in 6 studies (75%), and *Enterococcus* in 4 studies (50%). NGS methods yielded detection of *Streptococcus*, *Staphylococcus*, *Enterococcus*, and *Propionibacterium* in all 4 studies (100%), *Lactobacillus* in 3 studies (75%), and *Bifidobacterium* in 2 studies (50%). Deeper level sequencing, investigated at the species level, found no microbial patterns across all included studies.

Microbial Predominance

Included studies defined microbial predominance as the most abundant genera or species populating the human milk microbiota. The genera *Streptococcus* and *Staphylococcus* were

Table 1. Microbiota of Human Milk: Characteristics of Qualifying Studies.

Reference, Location	Research Question	Sample Size	Study Population	Collection and Storage	Analysis	Results: Observed Biodiversity Among Healthy Mothers	Conclusion
Pre-Next-Generation Sequencing							
Martín et al,[16] 2007, Spain	Evaluate the dominant bacteria in HM of mothers with vaginal and cesarean births	n = 4	Healthy mothers	Aseptically collected 7 days postpartum; icebox and then –80°C storage	PCR with DGGE[a]	Predominant: *Streptococcus* and *Staphylococcus; Pseudomonas* in vaginal deliveries	HM is a source of commensal bacteria for the infant gut.
Collado et al,[6] 2009, Spain	Characterize the HM microbiota	n = 50	Healthy mothers	HE and aseptically collected; –4°C and then –80°C storage	qRTi-PCR[b]	Predominant: *Bifidobacterium*, *Lactobacillus*, *Staphylococcus*, and *Streptococcus*	HM contains an abundance of bacterial DNA.
Cabrera-Rubio et al,[26] 2012, Finland	Identify factors influencing HM bacteria and compare bacteria of different body sites	n = 18	8 healthy and 10 overweight mothers	Aseptically collected within 2 days and at 1 and 6 months postpartum; –20°C storage	qPCR, 3 secondary PCRs/sample[c]	Predominant: *Weiselle* and *Leuconostoc;* increase in abundance of *Veillonella*, *Leptotrichia*, and *Prevotella* in 1- and 6-month milk samples	The HM microbiome changes over lactation stages and differs by maternal weight and delivery mode.
Collado et al,[22] 2012, Finland	Analyze maternal influences—namely, weight—on HM microbiota and cytokines	n = 56	34 normal-weight and 22 overweight mothers	HE aseptically collected at 24–48 hours, 1 and 6 months postpartum; –20°C storage	DNA extraction and qRTi-PCR[b]	No predominant genera observed; present: *Bifidobacterium* and *Streptococcus* were observed in 100% of 1- and 6-month milk samples[d]	HM is the single most important postpartum element in metabolic and immunological programming of the infant's health.
Tuzun et al,[12] 2013, Turkey	Evaluate the effect of breast milk jaundice development on HM and infant feces' microbiota	n = 60	30 healthy and 30 mothers with breast milk jaundice	HE and aseptically bilaterally collected between 1 and 28 days postpartum; first ice and then –70°C storage[e]	Real-time PCR[b]	Predominant: *Bifidobacterium*[e]	HM microbial content may play a role in breast milk jaundice.
Urbaniak et al,[17] 2014, Canada	Investigate chemotherapy's effects on HM's microbiota and metabolome (case study)	n = 9	8 healthy mothers and 1 undergoing chemotherapy	Aseptically collected over 4 months; initial ice and then –20°C storage	PCR and gas chromatography/ mass spectrometry[f]	No predominant genera observed; identified a wide microbial diversity in healthy mom's milk[e]	A wide microbial diversity was identified in healthy mothers' milk.
Khodayar-Parto et al,[21] 2014, Spain	Examine the effects of lactation stage, gestational age, and delivery mode on HM microbiota	n = 32	Healthy mothers, 13 with term and 19 with preterm deliveries	Mothers EE and collected HM; 3 samples taken at days 1–5, 6–15, and 17; –20°C storage	DNA extraction and qPCR[b]	*Bifidobacterium*, *Lactobacillus*, *Staphylococcus*, and *Enterococcus* identified in all mature milk samples from mothers with term and preterm deliveries	Lactation stage, degree of prematurity, gestational age, and delivery mode influence HM microbiota.
Olivares et al,[19] 2015, Spain	Describe how celiac disease alters HM composition and microbiota	n = 24	12 healthy mothers and 12 with celiac disease	Mothers HE and aseptically collected milk at early feed; –20°C storage	Conventional, qRTi-PCR CE-LIF[b]	Predominant: *Bifidobacterium*[d]	Mothers with celiac disease have a decreased abundance of immunoprotective compounds and certain bacteria than healthy mothers.

(continued)

Table 1. (continued)

Reference, Location	Research Question	Sample Size	Study Population	Collection and Storage	Analysis	Results: Observed Biodiversity Among Healthy Mothers	Conclusion
				Next-Generation Sequencing			
Hunt et al,[13] 2011, United States	Evaluate stability and diversity of HM bacteria over time	n = 16	Healthy mothers	Mother EE, aseptically collected 3 times over 4 weeks; –20°C storage	Pyrosequencing[c]	9 core genera OTUs present in all samples: *Staphylococcus*, *Streptococcus*, *Serratia*, *Pseudomonas*, *Corynebacterium*, *Ralstonia*, *Propionibacterium*, *Sphingomonas*, *Bradyrhizobium*	HM contains a diverse and complex bacterial community.
Jost et al,[10] 2013, Switzerland	Investigate bacterial diversity of HM of mothers who gave vaginal birth	n = 7	Healthy mothers	Aseptically collected at 3 time points; –80°C storage	Cultures, Sanger sequencing, 454-pyrosequencing, 16S ribosomal RNA gene sequencing[g]	Predominant: *Staphylococcus*, *Streptococcus*, and *Propionibacterium*	HM may significantly influence infant gut colonization and immune system.
Ward et al,[20] 2013, Canada	Examine the HM metagenome	n = 10	Healthy mothers	Aseptically collected unsterilized breast milk 9–30 days postpartum; pooled samples; –70°C storage	Illumina sequencing[b]	Predominant: *Pseudomonas*, *Staphylococcus*, and *Streptococcus*	Diversity of bacterial may be beneficial; benefits are not associated with particular genera or species.
Jiménez et al,[24] 2015, Spain	Investigate the effects of mastitis on HM's metagenome	n = 20	10 healthy mothers and 10 with mastitis	HE and aseptically collected	Cultures, shotgun libraries from 454-pyrosequenced DNA[b]	Predominant: *Bacteroides*, *Bifidobacterium*, *Burkholderia*, *Faecalibacterium*, *Lactobacillus*, *Novosphingobium*, *Propionibacterium*, *Pseudomonas*, *Ruminococcus*, *Sphingomonas*, *Sphingobium*, *Sphingopyxis*, *Staphylococcus*, and *Streptococcus*	Relative to healthy mothers, women with mastitis have different HM metagenomes.

CE-LIF, electrophoresis-laser-induced fluorescence; DGGE, denaturing gradient gel electrophoresis; EE, electric expression; HE, hand expression; HM, human milk; OTU, operational taxonomic unit; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; qRTi, quantitative real time.
[a]PCR amplification of the V6–V8 regions of 16S ribosomal RNA.
[b]Authors did not specify which hypervariable region was amplified.
[c]Targeted amplification of the V1 and V2 regions of 16S ribosomal RNA.
[d]Authors reported data on additional milk components.
[e]Authors reported additional data on feces components.
[f]Targeted amplification of the V6 region of 16S ribosomal RNA.
[g]Targeted amplification of the V5–V6 regions of 16S ribosomal RNA.

Table 2. Bacterial Diversity of Human Milk From Healthy Mothers Stratified by Sequencing Method.[a]

Genus	Species	Martín et al[16]	Collado et al[6]	Cabrera-Rubio et al[26]	Collado et al[22]	Tuzun et al[12]	Urbaniak et al[17]	Khodayar-Parto et al[21]	Olivares et al[19]	Hunt et al[13]	Jost et al[10]	Ward et al[20]	Jiménez et al[24]
		Pre-Next-Generation Sequencing								Next-Generation Sequencing			
Acinetobacter		y		y			y						
	calcoaceticus	y											
Actinomyces										y			
Akkermansia					y							y	
	muciniphila				y								
Anoxybacillus-Bacillus							y						
Atopobium							y						
Bacillus							y						
Bacteroides[b]			y										y[c]
Bifidobacterium			y[c]	y	y[d]	y[c]	y	y[e]	y[c]		y		y
	bifidum					y			y				
	breve								y		y		y
	fragilis								y				
	longum					y							y
Bradyrhizobium[f]										y[c]			
Brevundimonas-Caulobacter							y						
	diminuta						y						
Burkholderia[b]													y[c]
Campylobacter												y	
Candidatus												y	
Candidatus Phytoplasma													y
Carnobacterium				y									
Citrobacter				y									
Cloacibacterium							y						
Clostridium				y	y				y				
	coccoides			y	y				y				
	IV		y										
	XIVa		y										
	XIVb		y										
Corynebacterium[f]				y			y			y[c]		y	y
Edwardsiella												y	
Enterococcus		y	y	y	y			y[g]	y		y		y
	faecalis	y									y		

(continued)

Table 2. (continued)

Genus	Species	Martín et al[16]	Collado et al[6]	Cabrera-Rubio et al[26]	Collado et al[22]	Tuzun et al[12]	Urbaniak et al[17]	Khodayar-Parto et al[21]	Olivares et al[19]	Hunt et al[13]	Jost et al[10]	Ward et al[20]	Jiménez et al[24]
		Pre-Next-Generation Sequencing								Next-Generation Sequencing			
	faecium	y											
	gallinarum										y		
Escherichia		y									y		
	coli	y											
Eubacterium							y						
Faecalibacterium[b]							y						y[c]
	prausnitzii						y						
Finegoldia							y					y	
Flavobacterium-Cytophaga							y						
Gardnerella							y						
Gemella		y					y			y			
	haemolysans	y											
Granulicatella										y			
Klebsiella											y		
	pneumoniae										y		
Lactobacillus[b]		y	y[c]	y	y	y	y	y[g]	y		y	y	y[c]
	brevis										y		
	delbrueckii						y						
	fermentum					y							
	gasseri					y					y		
	plantarum	y				y							
	rhamnosus					y							
Lactococcus		y		y									
	lactis	y											
Leptotrichia				y									
Leuconostoc		y		y[c]									
	citreum	y											
Lysinibacillus				y									
Methylophilus							y						
Mycoplasma												y	
Neisseria													y
Novosphingobium													y
Pantoea												y	
Parabacteroides													y
Peredibacter							y						

(continued)

Table 2. (continued)

Genus	Species	Martín et al[16]	Collado et al[6]	Cabrera-Rubio et al[26]	Collado et al[22]	Tuzun et al[12]	Urbaniak et al[17]	Khodayar-Parto et al[21]	Olivares et al[19]	Hunt et al[13]	Jost et al[10]	Ward et al[20]	Jiménez et al[24]
		Pre-Next-Generation Sequencing								Next-Generation Sequencing			
Petrobacter							y						
Porphyrobacter							y						
Porphyromonas							y						
	synxantha	y											
Prevotella				y			y			y			
Propionibacterium[b,f]		y					y			y[c]	y[c]	y	y[c]
	acnes	y									y		
	granulosum										y		
Pseudomonas[f]		y[h]					y			y[c]		y[c]	y
Ralstonia[f]										y[c]			
Rhizobium-Agrobacterium							y						
Rothia							y			y	y		
	mucilaginosa										y		
Roseburia													y
Ruminococcus[b]							y						y[c]
	gnavus						y						
Schlegelella							y						
Serratia[f]		y								y[c]			
	proteamaculans	y											
Shigella											y		
Sphingobacterium							y						
Sphingomonas[f] *Sphingobium*							y			y[c]			y
Sphingopyxis													y
Staphylococcus[b,f]		y[c]	y[c]	y	y		y	y[e]		y[c]	y[c]	y[c]	y[c]
	aureus			y	y						y		
	epidermidis	y									y		
	haemolyticus										y		
	hominis	y									y		
	lugdunensis										y		
	pasteuri										y		
	salivarius	y											
	warneri										y		
Stenotrophomonas				y									
Streptococcus[b,f]		y[c]	y[c]	y	y[d]		y	y	y	y[c]	y[c]	y[c]	y[c]
	atypical-dispar-parvula										y		

(continued)

Table 2. (continued)

Genus	Species	Martín et al[16]	Collado et al[6]	Cabrera-Rubio et al[26]	Collado et al[22]	Tuzun et al[12]	Urbaniak et al[17]	Khodayar-Parto et al[21]	Olivares et al[19]	Hunt et al[13]	Jost et al[10]	Ward et al[20]	Jiménez et al[24]
		Pre-Next-Generation Sequencing								Next-Generation Sequencing			
	mitis	y									y		
	parasanguinis	y											
	pneumoniae	y											
	salivarius	y									y		
	thermophilus						y				y		
	vestibularis										y		
Thermus							y						
Veillonella		y		y							y		
Weissella		y		y[c]									
	cibaria	y											
	confusa	y											

[a]Genera are listed in alphabetical order; species are listed in alphabetical order by genus. y, genera or species identified as present.
[b]Core 7.[24]
[c]Genera or species identified as a predominant.
[d]Authors did not denote prevalent genera, but these genera were identified in 100% human milk samples measured at 1-month and 6-month milk samples among normal-weight mothers.
[e]Identified in 100% of samples of both term and preterm mature milk.
[f]Core 9. Hunt et al[13] misreported the family Bradyrhizobiaceae as a part of the core 9 genera, instead of the genus *Bradyrhizobium*, which we have classified as "core 9" above.
[g]Identified in 100% of samples from transitional and mature milk in both preterm and term.
[h]Identified as present in cesarean section birth but predominant in vaginal births.

predominant in 6 of the 12 formally evaluated studies (50%) (Table 2). *Bifidobacterium* was defined as predominant in 3 studies (25%), *Propionibacterium* in 3 studies (25%), *Lactobacillus* in 2 studies (17%), and *Enterococcus* in 0 studies (0%). Again, differences in methodological approaches were observed. Using a conventional PCR-based approach, *Streptococcus* and *Staphylococcus* were predominant in 2 studies (25%), *Bifidobacterium* in 3 studies (38%), *Propionibacterium* in 0 studies (0%), *Lactobacillus* in 1 study (13%), and *Enterococcus* in 0 studies (0%). Using NGS, *Streptococcus* and *Staphylococcus* were predominant in all 4 studies (100%), *Bifidobacterium* in 0 studies (0%), *Propionibacterium* in 3 studies (75%), *Lactobacillus* in 1 study (25%), and *Enterococcus* in 0 studies (0%). Deeper level sequencing, investigated at the species level, found no predominant species-specific microbial patterns.

Discussion

Studies included in this systematic review varied with respect to geographic location and methods of milk collection, storage, and analysis. *Streptococcus* and *Staphylococcus* were the predominant genera in 6 of the 12 studies (50%) and in all 4 (100%) of those using NGS methods. These 2 genera, *Streptococcus* and *Staphylococcus*, appear to be widely predominant in human milk without regard to differences in geographic location or analytic methods.

Our findings identified only 2 consistently predominant genera that contradict existing research reporting a "core 9" and "core 7" human milk microbiota.[13,24] Jiménez et al[24] and Hunt et al[13] identified two diverse core human milk microbiomes. Using shotgun amplification, Jiménez et al identified a healthy core human milk microbiome that included the genera *Staphylococcus*, *Streptococcus*, *Bacteroides*, *Faecalibacterium*, *Ruminococcus*, *Lactobacillus*, and *Propionibacterium*. At the species level, there was a high degree of interindividual variability observed among healthy women. Using NGS, Hunt et al identified a set of 9 operational taxonomic units that were present in all milk samples collected: *Staphylococcus*, *Streptococcus*, *Serratia*, *Pseudomonas*, *Corynebacterium*, *Ralstonia*, *Propionibacterium*, *Sphingomonas*, and *Bradyrhizobiaceae*; bacterial species were not identified.[13] Between these 2 studies, *Staphylococcus*, *Streptococcus*, and *Propionibacterium* were the only genera commonly reported as predominant in both identified core human milk microbiotas. This suggests that additional genera identified as a part of the "core 9" and "core 7" may not be consistently represented as predominant genera in the human milk microbiota. Identification of the "core 9" and "core 7" used culture-independent methods but analyzed mother's milk from different geographic locations with variable milk collection, storage, and analytic methods. Using NGS, Hunt et al amplified the V1–V2 hypervariable region of the 16S rRNA gene. Jiménez and colleagues did not specify which hypervariable region was amplified, but taxonomy was assigned based on shotgun sequencing. Despite the methodological differences in these studies, their mutual identification of *Streptococcus* and *Staphylococcus* as part of the "core" genera is consistent with our findings and suggests that these genera may be widely predominant in human milk.

The ability to detect bacterial diversity in human milk may be dependent on milk collection techniques and analytic methods. By cloning and sequencing DNA, PCR is an effective way to characterize microbial communities.[27] However, recent literature suggests that certain genera are better identified by primers targeting specific 16S variable gene regions.[29,30] Observed between-study differences may be attributable to variations in regional amplification. Relative to conventional rRNA PCR, NGS is a more sensitive and less biased analytic method for identifying and quantifying bacterial genera in human milk.[31] This suggests that the presence/predominance of *Streptococcus* and *Staphylococcus* may be underestimated in studies using conventional methods.

The phylogeny and biological significance of the human milk microbiota have been described in detail elsewhere.[8,28,32,33] The entero-mammary pathway suggests that microbes located in the maternal gut translocate to the mammary glands and, upon milk consumption, colonize the infant gut.[8,28,32,33] The human milk microbiota may be involved in the development of infant innate immunity and serve as a functional link between maternal and infant gut microbiota.[8,33] This relationship is multifactorial and dependent on many potential confounding factors such as delivery mode,[21,34–39] antibiotic use,[15,18] and maternal obesity.[22,26,40] Infants exclusively breastfed have been shown to have a less diverse and rich intestinal microbiota in comparison to infants formula fed.[41,42] This decreased diversity and richness may be attributed to microbes present in human milk and human milk oligosaccharides[41,42] potentially influencing the colonization of the infant gut. The infant gut microbiota has been associated with neurodevelopmental outcomes and may play a role in early brain development.[43] The human milk microbiota contains some of the first microbes to be introduced into the infant gut, thus potentially playing a large role in the colonization of the infant gut and development of the immune system.[26] Interventions designed to modify maternal gut microbiota (eg, diet, nutrition supplements) may affect human milk microbiota and subsequently influence infant gut microbiota and alter infant health outcomes.[33,44,45]

Our study has several strengths worth highlighting. Both reviewers independently conducted a PubMed search and evaluated qualifying manuscripts. This systematic approach, designed to maximize reliable results, ensured precise study identification and data abstraction processes. Our inclusion criteria were broad and not restricted to specific variations of culture-independent methods. This increased the generalizability of our findings, allowing a comparison of studies using both conventional PCR and NGS methods. By design, our study also included only those investigating the bacterial diversity of the human milk microbiota, thus enabling an unbiased synthesis of predominant bacterial diversity.

The observed results are subject to several limitations. First, reviewers were not blinded to the purpose of this study. This may have introduced a nondifferential bias, leading to either overinterpretation or underinterpretation of common present or predominant genera. Second, to maximize the abstracted study characteristics, only studies written in English were included in this systematic review. Third, to ensure all identified studies were peer-reviewed, we did not include published abstracts presented at previous academic meetings. Fourth, given the descriptive nature of our research question, we abstracted and evaluated study results but did not conduct formal evaluations of study design, methodological rigor, or research methods. This descriptive approach was purposeful in design to be more inclusive, thus allowing synthesis of studies that used either conventional PCR or NGS.

Conclusion

In summary, this systematic review of the literature reports that the genera *Streptococcus* and *Staphylococcus* are the predominant genera in the human milk microbiota. We suggest that these 2 genera may be universally predominant in the human milk microbiota, independent of geographic location or milk collection technique and may have been underestimated in previous work using conventional PCR methods. Future research to confirm these findings and to further clarify the effects of human milk microbes on infant short-term and long-term health outcomes should use NGS methods to maximize detectable bacterial diversity.

Statement of Authorship

J. L. Fitzstevens conceived and executed the study, abstracted data, drafted the manuscript, approved the final submission, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. K. C. Smith conceived and executed the study, abstracted data, reviewed and revised the manuscript, approved the final submission, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. J. I. Hagadorn conceived and executed the study, reviewed and revised the manuscript, approved the final submission, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. M. J. Caimano conceived and executed the study, reviewed and revised the manuscript, approved the final submission, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. A. P. Matson conceived and executed the study, reviewed and revised the manuscript, approved the final submission, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. E. A. Brownell conceived and executed the study, supervised the overall study and coordinated the interpretation of results, reviewed and revised the manuscript, approved the final submission, and agrees to be accountable for all aspects of work ensuring integrity and accuracy.

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