Published in "Journal of Infection 81(1): 17–47, 2020"
which should be cited to refer to this work.

Breast milk microbiota: A review of the factors that influence composition ☆

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Keywords:
Microbiome
Diversity
Delivery
Caesarean
GBS
Antibiotics
BMI
Probiotics
Smoking
Diet

SUMMARY

Breastfeeding is associated with considerable health benefits for infants. Aside from essential nutrients, immune cells and bioactive components, breast milk also contains a diverse range of microbes, which are important for maintaining mammary and infant health. In this review, we summarise studies that have investigated the composition of the breast milk microbiota and factors that might influence it.

We identified 44 studies investigating 3105 breast milk samples from 2655 women. Several studies reported that the bacterial diversity is higher in breast milk than infant or maternal faeces. The maximum number of each bacterial taxonomic level detected per study was 58 phyla, 133 classes, 263 orders, 596 families, 590 genera, 1300 species and 3563 operational taxonomic units. Furthermore, fungal, archaeal, eukaryotic and viral DNA was also detected. The most frequently found genera were *Staphylococcus, Streptococcus Lactobacillus, Pseudomonas, Bifidobacterium, Corynebacterium, Enterococcus, Acinetobacter, Rothia, Cutibacterium, Veillonella* and *Bacteroides.* There was some evidence that gestational age, delivery mode, biological sex, parity, intrapartum antibiotics, lactation stage, diet, BMI, composition of breast milk, HIV infection, geographic location and collection/feeding method influence the composition of the breast milk microbiota. However, many studies were small and findings sometimes contradictory. Manipulating the microbiota by adding probiotics to breast milk or artificial milk offers an exciting avenue for future interventions to improve infant health.

Introduction

Human breast milk (BM) is the optimal nutrition for infants. Aside from essential nutrients (proteins, fats, carbohydrates, vitamins and minerals), BM also contains a broad range of immune cells and bioactive components that have anti-inflammatory, anti-infective and probiotic actions.[1,2] These include antimicrobial peptides (such as bacteriocin, lactoferrin, lysozyme, lactadherin), cytokines, chemokines, immunoglobulins, growth factors, oligosaccharides, glycoconjugates and fatty acids. Breastfeeding is associated with considerable health benefits including protection from diarrhoea,[3–5] necrotising enterocolitis,[6,7] respiratory infections[5,8–11] (including acute otitis media),[8,9] oral candiasis,[9] enterovirus infection,[12] atopic dermatitis,[4] obesity[13,14] and allergic disease.[15] These benefits are especially relevant to infants with increased susceptibility, such as preterm or sick infants. Increasing evidence shows that BM contains its own microbiota, which likely has important health implications for both mothers (mammary gland health) and infants (intestinal colonisation and protection against pathogens, maturation of the immune system and digestion of nutrients).

Microbial colonisation of infants increases rapidly after birth and the maternal microbiota is the main source for the infant microbiota.[16] The importance of the maternal BM microbiota is underlined by the differences in the intestinal microbiota between breastfed and formula-fed infants.[16] Exclusively breastfed infants have a lower diversity in their intestinal microbiota with a higher relative abundance of *Bifidobacterium* (and higher number of different *Bifidobacterium* species), *Staphylococcus* and *Streptococcus*, while formula-fed infants have a higher relative abundance of *Bacteroides, Clostridium, Enterobacteriaceae, Enterococcus* and *Lachnospiraceae.*[16] Furthermore, there is a correlation between mother-infant pairs in BM and infant's faeces in the relative abundance of *B. adolescentis, B. bifidum, B. breve* and *Lactobacillus plantarum.*[17–19] The composition of the intestinal microbiota is crucial for the development of the immune system and disruption has been associated with adverse health outcomes later in life, including

☆ PZ drafted the initial manuscript. NC critically revised the manuscript and both authors approved the final manuscript as submitted.

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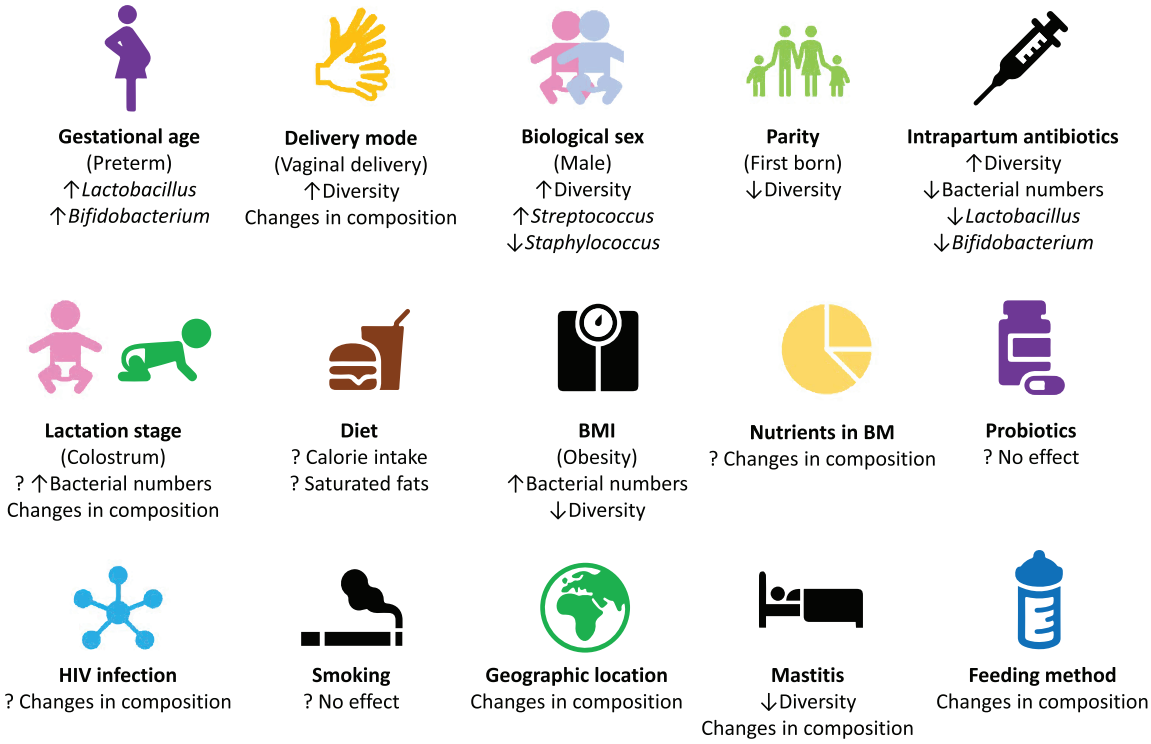

Fig. 1. Factors that have been reported to influence the composition of the intestinal microbiota.

the development of allergic disease,[20] chronic inflammatory bowel diseases,[21] obesity,[22] diabetes mellitus[23] and diminished vaccine responses.[24]

Currently, little is known about the composition of the BM microbiota and even less about the factors that determine it. In this review, we summarise studies that have investigated the BM microbiota in humans and intrinsic and extrinsic factors that might influence it. Understanding this will help maximise the benefits of breastfeeding and to potentially optimise breast milk or artificial nutrition by the addition of beneficial microbes or microbial DNA. Organisms detected in BM might also be candidates for the development of probiotics.

Systematic review methods

In August 2019, MEDLINE (1946 to present) was searched using the Ovid interface with the following search terms: (breastfeeding OR breast feeding OR breast milk OR human milk OR colostrum OR lactation / im) AND (bacteria OR microbio* OR metagenom* (metagenome)) OR milk Human / mi [Microbiology] OR colostrum / mi [Microbiology]. Only original studies investigating the microbial composition of human BM were included. Exclusion criteria were: (i) studies investigating selected components rather than the overall composition of the BM microbiota; (ii) those investigating pasteurised milk; and (iii) those that did not use culture methods that support the growth of commensal organisms. References of retrieved articles were hand-searched for additional publications. The selection of included studies is summarised in Fig. 1.

The following variables were extracted from the selected articles: year of study, country, number and characteristics of participants (including age of participants, previous antibiotic treatment, probiotic administration, gestational age, delivery mode, feeding method), collection method, timing of BM collection in relation to delivery, microbiota analysis method and key findings (including changes in diversity, abundance of microbes and association with different factors).

Results

Our search identified 770 studies. Of these, 41 fulfilled the inclusion criteria. An additional 3 relevant studies were identified by hand-searching of references. The 44 studies included in this review investigated 3105 BM samples from 2655 women.[19,25–67] The number of participants in each study ranged from 7 to 554 (median 32, mean 62) and the number of samples ranged from 10 to 554 (median 47, mean 72). All identified studies were case series and therefore at high risk for bias. Several studies were excluded, because the overall composition of the BM was not assessed,[18,68–75] BM was pasturised[76], they focused on the detection of potential pathogens[77–87], or did not provide enough details about the samples.[88]

The results of the studies are summarised in Table 1 and Fig. 1. The studies were done in 20 different countries (Spain 10, Finland 5, USA 4, Canada 3, China 2, Italy 2, Switzerland 2, Taiwan 2, Brazil 1, Haiti 1, India 1, Ireland 1, Norway 1, Malaysia 1, Mexico 1, Mozambique 1, Slovenia 1, South Africa 1, Syria 1, China and Taiwan 1, Burundi and Italy 1, China, Finland, South Africa and Spain 1). Multiple methods were used to determine the microbiota, including bacterial culture 11, polymerase chain reaction (PCR) 12, PCR-denaturing gradient gel electrophoresis (DDGE) 2, matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF-MS) 3, 16S rRNA gene sequencing 28 and metagenomic shotgun sequencing 3. Fourteen studies used two different methods and one study three different methods. To illustrate the heterogeneity in microbiota analysis, details for of the 27 studies that used 16S rRNA gene sequencing are summarised in Table 2. In total, 21 different DNA extraction kits, 5 different sequencing machines (Illumina HiSeq 2, Illumina MiSeq 14, Genome Sequencer FLX 6, Ion Torrent 3, sequencing machine not specified 3), 9 different hypervariable regions (V1-V2 4, V1-V3 2, V2-V3 1, V3-V4 7, V4 7, V5-V6 1, V6 1, V6-V8 1, V2-4-8 and V3-6, 7-9 2, region not specified 2), 13 different primers pairs and 5 different databases (Greengenes Database 13, Ribosomal Database Project

Table 1
Summary of findings of studies investigating the breast milk microbiota.

Author Country Publication year	No of women, no of samples Mean maternal age±SD (range) Maternal antibiotic use Maternal probiotic use Mean gestational age±SD (range) Delivery mode Feeding method Method of BM collection	Microbiota analysis technique (incl. DNA extraction kit, region, primers, PCR cycles, machine and database for sequencing)	Timing of testing after delivery	Main bacterial genera* (relative abundance unless otherwise stated) *families if genera not reported	Number of taxa Number of microbes	Phyla, species and other findings
MALDI-TOF-MS and shotgun sequencing						
Pärnänen et al.[25] Finland 2018	16, 32 Maternal age ns 50% received IAP (penicillin 7, cephalothin 1) 36% received probiotics (*B. longum* BB536, L. *paracasei* ST11 or L. *rhamnosus* LGG2) Term infants 100% delivered vaginally Feeding method ns* Cleaning breasts with water and soap, manual expression, discarding first drops	Shotgun sequencing InviMag Faeces DNA kit Illumina NextSeq SILVA Database Strainphlan	1–7d 1m	*Bifidobacterium* *Lactobacillus* *Staphylococcus* *Blautia* *Rothia* *Escherichia* *Eubacterium* *Akkermansia* *Bacteroides* *Ruminococcus* *Streptococcus* *Subdoligranulum*	Ns	• Overlap of mobile genetic elements in BM and infant faeces
Damaceno et al.[26] Brazil 2017	47 (2 mastitis), 141 25±6y (range ns) 6% received AB during pregnancy, 4% during lactation Probiotic use ns Gestational age ns 57% delivered vaginally Feeding method ns* Cleaning breasts with chlorhexidine, further collection method ns	MALDI-TOF-MS	1d 5–9d 25–30d	*Staphylococcus* (83%) *Streptococcus* (7%) *Lactobacillus* (4%) *Corynebacterium* (5%) *Actinomyces* (<1%) *Bifidobacterium* (<1%)	Bacteria: 16 species $10^{1.5-4}$ CFU/ml $10^{3.9}$ CFU/ml colostrum	• Total number of bacteria higher in colostrum than mature milk • *S. epidermidis* > *S. lugdunensis* > *L. gasseri, S. salivarius* > *S. caprae, C. tuberculostearicum* > *S. aureus, S. hominis, S. mitis, C. kroppenstedtii, S. sacharolyticus, S. capitis, S. parasanguinis, S. agalatiae, A. neuii, B. breve* • L. *gasseri* only detected in women with normal weight and who delivered vaginally

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Table 1 (*continued*)

Jiménez et al.[27] Spain 2015	20 (10 healthy, 10 mastitis), 20 Maternal age ns No AB previous 3 m No probiotics previous 3 m Gestational age ns Delivery mode ns Feeding method ns* Cleaning breasts with sterile water, manual expression with sterile gloves, discarding first drops	MALDI-TOF-MS Shotgun sequencing No use of DNA extraction kit Genome Sequencer FLX MG7 program	ns	*Pseudomonas* *Staphylococcus* *Sphingomonas* *Bacteroides* *Novosphingobium* *Sphingobium* *Sphingopyxis* *Streptococcus* *Methylobacterium* *Ruminococcus* *Roseburia* *Faecalibacterium* *Eubaterium* *Parabacteroides* *Enterococcus* *Lactobacillus* *Bifidobacterium* *Bukholderia* *Neisseria* *Corynebacterium* *Cutibacterium*	Bacteria: 12 phyla, 275 species Fungi: 2 phyla, 10 species Bacteria: 10^3 CFU/ml 22–152 bacterial species/sample 1–5 fungal species/sample	• Bacterial phyla: Proteobacteria, Firmicutes, Bacteroides, Actinobacteria, Tenericutes, Chloroflexi, Verrucomicrobia, Deinococcus-Thermus, Fibrobacteres, Cyanobacteria, Chlorobi, Acidobacteria • High absolute abundance of *S. aureus* in women with acute mastitis and *S. epidermidis* in women with subacute mastitis • Fungal DNA detected in 85% (17/20) of samples. *Malassezia globosa* most abundant. Other fungi: *Calocera cornea, Guepiniopsis buccina, Podospora anserine, Sordaria macrospora, Candida dubliniensis, M. restricta, Talaromyces stipitatus, Yarrowia lipolytica* • Eukaryotic DNA detected in 100% (20/20) of samples. *Toxoplasma gondii* in 35% (7/20) and *Giardia lamblia* in <1% (1/20) of samples. Other eukaryotes: *Dicyostelium discoideum, D. purpureum, Paramecium tetraurelia* • Archaeal DNA detected in 80% (8/10) of healthy women and none of the women with mastitis. *Haloarcula marismortui, H. utahensis, H. muko* • Viral DNA detected in 100% (20/20) of samples. *Betapapillomavirus, Cytomegalovirus, Lentivirus, Simplexvirus, Staphylococcus* phage, human endogenous retroviruses
Ward et al.[28] Canada 2013	10, 10 Maternal age ns AB use ns Probiotics use ns Gestational age ns Delivery mode ns Feeding method ns* Breasts not cleaned or sterilised, manual expression or by pump	Shotgun sequencing No use DNA extraction kit Illumina GAIIx Genome Analyser and Illumina CASAVA analysis pipeline MG-RAST pipeline	9–30d	*Staphylococcus* (75%) *Pseudomonas* (15%) *Edwardsiella* (2%) *Pantoea* (1%) *Treponema* (1%) *Streptococcus* (1%) *Campylobacter* (1%)	Bacteria: 360 genera	• Bacterial phyla: Proteobacteria (65%), Firmicutes (34%)

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Albesharat et al.[29] Syria 2011	15, 15 Maternal age ns No antibiotics during sampling Probiotic use ns Term infants 100% delivered vaginally Feeding method ns* Cleaning breasts with sterile water, manual expression with sterile gloves, discarding first drops	MALDI-TOF-MS 16S rRNA gene sequencing E.Z.N.A. bacterial DNA kit Primers 609F, 616R Sequencing machine ns Database ns RAPD-PCR	ns	*Enterococcus* *Lactobacillus* *Pediococcus* *Streptococcus*	Bacteria: 10^1–3×10^3CFU/sample (culture)	• Identical RAPD genotypes of L. *plantarum*, L. *fermentum*, L. *brevis*, *E. faecium*, *E. faecalis* and *P. pentosaceus* in faeces of women, BM and in infant faeces • *E. durans*, *E. faecium*, *E. faecalis*, *E. hirae*, *E. mundtii*, L. *animalis*, L. *brevis*, L. *fermentum*, L. *gasseri*, L. *helveticus*, L. *oris*, L. *plantarum*, *P. pentosaceus*, *S. australis*, *S. gallolyticus*, *S. vestibularis*
16S rRNA gene sequencing						
Hermansson et al.[30] Finland 2019	84, 84 32y (23–40) 36% received IAP (penicillin 18, cephalothin 5, cephalexin 1, penicillin plus metronidazole 1, cefuroxime plus metronidazole 3, cefuroxime 1) 73% received probiotics during pregnancy (L. *rhamnosus* LPR and *B. longum* BL999 or L. *paracasei* ST11 and *B. longum* BL999) 39.8 w (35.7–42.4) 73% delivered vaginally 70% exclusively breastfed Cleaning breasts, manual expression, discarding first drops	16S rRNA gene sequencing InviMag Faeces DNA kit V3-V4 Primers 515F, 806R PCR cycles ns Illumina MiSeq Greengenes Database	1m	*Streptococcaceae* *Staphylococcaceae* *Oxalobacteraceae* *Moraxellaceae* *Rhizobiales* *Gemellaceae* *Comamonadaceae* *Micrococcaeae*	Bacteria: 15 phyla, 54 families	• Bacterial phyla: Actinobacteria, Acidobacteria, Armatimonadetes, Bacteroidetes, Chlamydiae, Elusimicrobioa, Firmicutes, Fusobacteria, Planctomycetes, Proteobacteria, Spirochaetes, Thermi, TM7, Verrcomicrobia, WPS2 • Mode of delivery (strongest effect) and IAP affected the composition of the BM microbiota • Alpha-diversity and richness higher in women who delivered vaginally and in women who received IAP • *Bifidobacterium* not found in women who received IAP • Probiotics during pregnancy did not affect the composition of the BM microbiota
Ojo-Okunola et al.[31] South Africa 2019	554, 554 25y (19–40) 9% HIV infected Antibiotic use ns Probiotic use ns 23% preterm infants 81% vaginally delivered 53% exclusively breastfed Cleaning hands and breasts with soap and water, manual expression, discarding first drops	16S rRNA gene sequencing ZR Fungal/Bacterial DNA Miniprep™ V4 Primers 515F, 806R 30 PCR cycles Illumina MiSeq Ribosomal Database Project	6–10w	*Streptococcus* (49%) *Staphylococcus* (18%) *Rothia* (6%) *Corynebacterium* (4%) *Veillonella* *Gemella* *Acinetobacter* *Micrococcus*	Bacteria: 58 phyla, 133 classes, 263 orders, 596 families, 1300 genera, (core 9 genera)	• Bacterial phyla: Firmicutes (71%), Actinobacteria (16%), Proteobacteria (10%), Cyanobacteria (0.1%)

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Table 1 (*continued*)

Ding et al.[32] China 2019	89, 89 Mean maternal age ns (20–35y) AB use ns Probiotic us ns 94% term infants 52% delivered vaginally Feeding method ns* Cleaning breasts with sterile water, manual expression with sterile gloves, discarding first drops	16S rRNA gene sequencing Ezup Column Bacteria Genomic DNA Purification kit V3-V4 Primers ns 24 PCR cycles Illumina MiSeq Ribosomal Database Project qPCR	ns	*Staphylococcus* (100% of samples) *Bacillus* (87%) *Enterococcus* (76%) *Streptococcus* (76%) *Lactobacillus* (40%) *Pantoea* *Finegoldia* *Bifidobacterium* *Cutibacterium* *Gemella* *Anaerosalibacter* *Achromobacter* *Acinetobacter* *Sphingobium* *Raoultella* *Azospira* *Azospirillum* *Aeromonas* *Yersinia* *Lactococcus* *Duganella* *Hydrocarboniphaga* *Vulcaniibacterium* *Acidovorax*	Bacteria: 3 phyla, 6 classes, 14 orders, 23 families, 28 genera, 6 species, 93 OTUs (97% sequence similarity)	• Vaginal delivery associated with lower relative abundance of *Staphylococcus* and *Enterococcus*, but higher relative abundance of *Streptococcus* and *Lactobacillus* • High BMI associated with higher relative abundance of *Staphylococcus* and lower relative abundance of *Lactobacillus* and *Streptococcus* • Regional differences in diversity and composition of the BM microbiota
Moossavi et al.[33] Canada 2019	393, 393 Maternal age ns Antibiotic use ns Probiotic use ns Gestational age ns 76% delivered vaginally 41% exclusively breastfed Breasts not cleaned or sterilised, manual expression or by pump	16S rRNA gene sequencing Quick-DNA Fungal/Bacterial extraction kit V3-V4 Primers 515F, 806R PCR cycles ns Illumina MiSeq Greengenes Database	3–4m	*Streptococcus* (16%) *Ralstonia* (5%) *Staphylococcus* (5%)	Bacteria: 18 phyla	• Bacterial phyla: Proteobacteria (67%), Firmicutes (26%), Actinobacteria (4%), Bacteroidetes (1%) • Male sex, CS, indirect breastfeeding (pumped milk) and being first born independently associated with lower bacterial diversity • Maternal ethnicity, smoking, BMI, oligosaccharides concentration did not influence diversity of the BM microbiota • Higher relative abundance of *Gemellaceae, Vogesella* and *Nocardioides* with manually expressed milk, higher relative abundance of *Enterobacteriaceae* and *Pseudomonas* and lower relative abundance of *Bifidobacterium* in pumped milk • IAP, smoking, atopy, parity, mode of delivery did not influence the composition of the BM microbiota

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Simpson et al.[34] Norway 2018	125, 142 31±4y (range ns) AB use ns 46% received probiotics (1 m before until 3 m after delivery, L. *rhamnosus* GG, L. *acidophilus* La-5 and *B. animalis* ssp. *lactis* Bb-12) Gestational age ns 84% vaginally delivered Feeding method ns* Breasts not cleaned or sterilised, collection method ns	16S rRNA gene sequencing LGC Mag DNA extraction kit V3-V4 Primers Uiv_F, Univ_r 40–50 PCR cycles Illumina MiSeq Ribosomal Database Project	10d 3m	*Streptococcus* (100% of samples at d10; 96% at 3 m) *Staphylococcus* (87%; 71%) *Gemellaceae* (60%; 63%) *Rothia* (17%; 50%) *Veillonella* (17%; 40%) *Acinetobacter* (18%; 26%) *Haemophilus* (10%; 16%) *Bacillaceae* (10%; 17%) *Granulicatella* (ns; 26%) *Methylobacterium* (ns; 15%) *Klebsiella* *Lactobacillus* *Alkanindeges* *Stenotrophomonans* *Caulobacteraceae*	Bacteria: 69 genera	• Lower diversity and higher relative abundance of *Staphylococcus* and lower relative abundance of *Rothia, Granulicatella, Veillonella,* and *Methylobacterium* at 10d compared with 3 m • Administered probiotic bacteria found in BM,but had no effect on the composition of the BM microbiota
Tuominen et al.[35] Finland 2019	39, 35 Maternal age ns AB use ns Probiotic use ns 39.9 w (35.4–42.2) 62% delivered vaginally Feeding method ns* Collection method ns	16S rRNA gene sequencing High-salt method V3-V4 Primers ns Cycles ns Illumina MiSeq Greengenes Database	1d (31) 2m (4)	*Staphylococcus* *Streptococcus* *Veillonella* *Rothia* *Cutibacterium* *Haemophilus* *Corynebacterium* *Spingomonas* *Pseudomonas* *Prevotella* *Neisseria* *Lysinibacillus* *Lactobacillus* *Delftia* *Burkholderia* *Agrobacterium* *Actinomyces*	Bacteria: 18 genera	• Bacterial phyla: Firmicutes (80%), Proteobacteria (14%), Actinobacteria (5%), Bacteroides (0.5%)

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Li et al.[36] China Taiwan 2017	133 (102 China, 31 Taiwan), 133 29±5y (21–42) AB use ns Probiotic use ns Gestational age ns 39% delivered vaginally Feeding method ns* Cleaning breasts with alcohol with sterile gloves, expression by pump, discarding first drops	16S rRNA gene sequencing QIAquick PCR purification kit V1-V2 Primers 27F, 228R 15–20 PCR cycles Genome Sequencer FLX Greengenes Database	6.1 ± 4m	*Streptococcus* (24%) *Pseudomonas* (14%) *Staphylococcus* (12%) *Lactobacillus* (5%) *Cutibacterium* (2%) *Herbaspirillum* (2%) *Rothia* (2%) *Stenotrophomonas* (2%) *Acinetobacter* (2%) *Bacteroides* (1%) *Halomonas* (1%) *Veillonella* (0.9%) Spingomonas (0.9%) *Delftia* (0.8%) *Corynebacterium* (0.7%) *Micrococcus* (0.7%) *Bifidobacterium* (0.6%) Other (28%)	Bacteria: 16 phyla, 40 classes, 71 orders, 134 families, 245 genera, 98 species, 3563 OTUs (97% sequence similarity) 32–260 species/sample	• 4 *Lactobacillus* species: L. *paracasei*, L. *reuteri*, L. *rhamnosus*, L. *vaginalis* • 5 *Bifidobacterium* species detected: *B. adolescentis, B. dentium, B. longum, B. longum* susp. *Infantis, B. stercoris* • Delivery mode did not influence diversity, but women who delivered by CS had a higher relative abundance of *Lactobacillus* and unclassified OTUs • Geographic differences in the composition of the BM microbiota • Lactation stage (<3 m, 3–6 m, >6 m) had no influence on the BM microbiota • Maternal BMI had no influence on the BM microbiota
Toscano et al.[37] Italy 2017	29, 29 Maternal age ns No antibiotic during lactation Probiotic use ns Gestational age ns 52% delivered vaginally Feeding method ns* Cleaning hands and breasts with soap, further collection method ns	16S rRNA gene sequencing Milk DNA extraction kit V2–4–8 and V3-6, 7–9 Primers ns 30 PCR cycles Ion Torrent Personal Genome Machine Greengenes Database	< 3d	*Staphylococcus* (vaginally delivered 61%; CS 73%) *Streptococcus* (59%; 30%) *Prevotella* (2%; 9%) *Halomonas* (3%; 8%) *Finegoldia* (<1%; 4%) *Haemophilus* (4%; <1%) *Pseudomonas* (<1%; <1%)	ns	• Women who delivered vaginally had higher relative abundance of *Streptococcus* and *Haemophilus*, but lower relative abundance of *Finegoldia, Halomonas, Prevotella, Pseudomonas* and *Staphylococcus* • Women who delivered by CS had a higher number of environmental bacteria • Colostrum of women who delivered vaginally had a higher bacterial diversity compared with women who delivered by CS (not significant)
Pannaraj et al.[38] USA 2017	107, 133 Maternal age ns 31% IAP Probiotic use ns Term infants 65% delivered vaginally 52% exclusively breastfed Collection method ns	16S rRNA gene sequencing BiOstic Bacteremia DNA isolation kit V4 Primers 340F, 806R PCR cycles ns Illumina MiSeq Greengenes Database	1–331d	*Moraxellaceae* *Staphylococcaceae* *Enterobacteriaceae* *Pseudomonadaceae* *Streptococcaveae* *Weeksellaceae* *Xanthomonadaceae* *Gamellaceae* *Alicyclobacillaceae* *Bradyrhizobiaceae* *Caulobacteraceae* *Neisseriaceae*	ns	• No change in alpha-diversity over time; beta-diversity increased within first 6 m of life and then decreased • The following genera were detected in the BM and infant intestinal microbiota: *Actinobacillus, Actinomyces, Aggregatibacter, Atopobium, Bifidoabacterium, Bradyrhizobium, Bulleidia, Chryseobacterium, Clostridium, Haemophilus, Lactobacillus, Megamonas, Pseudomonas, Rothia, Scardovia, Streptococcus, Veillonella*

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Williams et al.[39] USA 2017	21, 104 30±4y (range ns) No antibiotics during collection period Probiotic us ns Gestational age ns Delivery method ns Feeding method ns* Breasts not cleaned or sterilised, expression by pump	16S rRNA gene sequencing QIAamp DNA faeces mini kit V1-V3 Primers 27F, 534R 20 PCR cycles Illumina MiSeq Ribosomal Database Project	2d 5d 10d 1m 2m 3m 4m 5m 6m	*Streptococcus* (d2 32%; 6 m 41%) *Staphylococcus* (33%; 21%) *Gemella* (13%; 0.7%) *Veillonella* (0.4%; 6%) *Rothia* (1%; 3%) *Lactobacillus* (only 1m-5 m) *Cutibacterium* (2%; 0.6%) *Corynebacterium* (1%; 3%) *Granulicatella* (0.3%; 2%) *Pseudomonas* (0.2%; 0.2%) *Prevotella* (2%; 0.4%) *Actinomyces* (0; 1%) *Clostridium* (0.1%; 0.1%) *Neisseria* (0.1%; 2%) *Bifidobacterium* (only d10, 2 m, 3 m) *Haemophilus* (0.3%; 0.1%)	Bacteria: 12 phyla, 16 genera	• Bacterial phyla: Firmicutes (85%), Actinobacteria (6%), Protebacteria (2%), Bacteroidetes (1%) • Higher relative abundance of *Veillonella* and *Granulicatella* at 6 m • Women with higher BMI had higher relative abundance of *Granulicatella* and lower relative abundance of *Bacteroides* • Energy consumption was positively associated with the relative abundance of *Granulicatella* • Higher SFA and MUFA intake was associated with lower relative abundance of *Corynebacterium*, higher protein intake with higher relative abundance of *Gemella* and higher fibre intake with *Rothia* • Women of male infants had higher relative abundance of *Streptococcus* and lower relative abundance of *Staphylococcus* in their BM • No association of the composition of the BM and maternal age, parity or delivery mode

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Biagi et al.[40] Italy 2017	36, 36 Maternal age ns No antibiotics No probiotics Term infants 100% delivered vaginally 100% exclusively breastfed Cleaning breasts with soap and water, expression by pump	16S rRNA gene sequencing DNeasy Blood&Tissue kit V3-V4 Primers S-D-Bact-0341-b-S-17, S-D-Bact-0785-a-A-21 25 cycles Illumina MiSeq Greengenes Database	20d	*Verucomicrobiaceae* *Pseudomonoadaceae* *Moraxellaceae* *Enterobacteriaceae* *Oxalobacteraceae* *Sphingomonadaceae* *Erysipelotrichaceae* *Veillonellaceae* *Ruminococcaceae* *Peptostreptococcaceae* *Lachnospiraceae* *Clostridiaceae* *Streptococcaceae* *Lactobacillaceae* *Enterococcaceae* *Gemellaceae* *Staphlyococcaceae* *Bacillaceae* *Glavobacteriaceae* *Prevotellaceae* *Porphyromonadaceae* *Bacteroidaceae* *Coriobacteriaceae* *Bifidobacteriaceae* *Cutibacteriaceae* *Micrococcaceae* *Corynebacteriaceae* *Acintomycetaceae*	Bacteria: 28 families, 69 OTUs (97% sequence similarity)	• BM microbiota more diverse than infant oral or faeces microbiota

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Table 1 (*continued*)

Drago et al.[41] Burundi Italy 2017	50 (30 Burundi, 20 Italy), 82 Italy 36±6y (range ns) Burundi 23±5y (range ns) 18% received IAP, no AB during lactation Probiotic use ns Italy 39.2 ± 1.4 w Burundi 37.2 ± 1.2 w 82% delivered vaginally Feeding method ns* Cleaning of hands and breasts with soap, further collection method ns	16S rRNA gene sequencing Milk DNA extraction kit V2–4–8 and V3-6, 7–9 Primers ns PCR cycles ns Ion Torrent Personal Genome Machine Greengenes Database	<3d 1m	*Achromobacter* *Acinetobacter* *Alcaligenes* *Arthrobacter* *Bacteroides* *Corynebacterium* *Cutibacterium* *Delftia* *Enterococcus* *Flavobacterium* *Gemella* *Halomonas* *Klebsiella* *Leuconostoc* *Pantoea* *Prevotella* *Pseudomonas* *Rhizobium* *Rhodanobacter* *Rothia* *Serratia* *Staphylococcus* *Streptococcus*	Bacteria: >200 genera/sample	• Higher relative abundance of anaerobic bacteria in mature milk compared with colostrum • Italy: predominance of *Abiotrophia* and *Alloiococcus* in colostrum and *Parabacteroides* mature milk • Burgundy: predominance of *Aquabacterium, Serratia* and *Peptrostreptococcus* in colostrum and *Rhizobium, Dolosigranulum* and *Weissella* in mature milk • No influence of diet on the composition of the BM microbiota
Cacho et al.[42] USA 2017	12, 12 27±5y (range ns) 92% received AB Probiotics use ns 27±3 w (range ns) 58% delivered vaginally Feeding method ns* Hand hygiene, expression by pump, 25% breastfed before taking sample	16S rRNA gene sequencing PowerFecal® DNA isolation kit V4 Primers 515F, 806R PCR cycles ns Illumina HiSeq Greengenes Database Culture	7–8m	*Halomonas* (26%) *Acinetobacter* (4%) *Staphylococcus* (15%) *Bacillus* (<1%) *Stenotrophomonas* (3%) *Streptococcus* (<1%) *Shewanella* (<8%) *Pseudomonas* (1%) *Serratia* (2%) *Enterococcus* (1%) *Bacteroides* (4%) *Agrobacterium* (<1%) *Corynebacterium* (6%) *Lactobacillus* (2%) *Chryseobacterium* (<1%)	Bacteria: up to 10^6/ml	

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Table 1 (*continued*)

Murphy *et al*[19] Ireland 2017	10, 10 (1 mastitis) Maternal age ns 10% received AB during lactation Probiotic use ns 39.1 ± 0.9 w (range ns) 60% vaginally delivered Feeding method ns* Cleaning breasts with chlorhexidine wearing sterile gloves, manual expression, discarding first drops	16S rRNA gene sequencing QIAamp DNA faeces mini kit V3-V4 Primers ns 30 PCR cycles Illumina MiSeq BLAST Database Culture	1w 3w 6w 3m	*Staphylococcus* (1w 12%; 3m 1%) *Streptococcus* (10%; 7%) *Pseudomonas* *Elizabethkingia* *Variovorax* *Bifidobacterium* (3%; 2%) *Flavobacterium* *Lactobacillus* *Stenotrophomonas* *Brevundimonas* *Chryseobacterium* *Enterobacter*	Bacteria: 207 genera (core 12 genera representing 81%)	• Bacterial phyla: Proteobacteria (41%), Firmicutes (35%), Bacteroidetes (17%) • Increase in bacterial richness from 3 to 6 w and decrease in richness and diversity from 6 to 12 w • BM microbiota more diverse than infant faeces microbiota
Patel et al.[43] India 2017	50 (32 mastitis), 50 26±2y (range ns) No antibiotics prior to collection No probiotics Gestational age ns Delivery mode ns Feeding method ns* Cleaning hands with soap, cleaning breasts with ethyl alcohol, manual expression with sterile gloves, discarding first drops	16S rRNA gene sequencing QIAamp fast DNA faeces mini kit V2-V3 Primers 101F, 518R 25 PCR cycles Ion Torrent Personal Genome Machine Greengenes Database	12–30d	*Acinetobacter* *Klebsiella* *Staphylococcus* *Pseudomonas* *Ralstonia* *Bacillus* *Aeromonas* *Erwinia* *Clostridium* *Leclercia* *Cronobacter* *Ruminococcus* *Lactobacillus* *Cutibacterium* *Corynebacterium* *Paenibacillus* *Pyramidobacter* *Hespellia* *Prevotella* *Eubacterium* *Pantoea* *Proteus* *Veillonella* *Leptospira* *Brevibacillus* *Methylobacterium* *Faecalibacterium* *Serratia* *Enterococcus* *Edwardsiella* *Burkhoderia*	Bacteria: 25 phyla, 185 families, 590 genera	• Bacterial phyla: Proteobacteria (50%), Firmicutes (17%), Actinobacteria, Spriochaetes, Synergistetes, Tenericutes, Bacteroidetes • Lower diversity, lower relative abundance of anaerobic bacteria, *Acinetobacter, Ruminococcus, Clostridium, Faecalibacterium* and *Eubacterium* and higher relative abundance of *Aeromonas, Staphylococcus, Ralstonia, Klebsiella, Serratia, Enterococcus* and *Pseudomonas* in women with mastitis

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Table 1 (*continued*)

Boix-Amoros et al.[44] Spain 2016	21, 30 Maternal age ns AB use ns Probiotic use ns Gestational age ns Delivery mode ns 100% exclusively breastfed Cleaning breasts with sterile water, soaked in chlorhexidine, manual expression, discarding first drops	16S rRNA gene sequencing MasterPure complete DNA and RNA purification kit Primers 8F, 785R 20 PCR cycles Genome Sequencer FLX Ribosomal Database Project qPCR (fusA gene)	<5d 6–15d 15–30d	*Staphylococcus* (80% of samples) *Streptococcus* (43%) *Finegoldia* (30%) *Pseudomonas* (27%) *Acinetobacter* (23%) *Anaerococcus* (17%) *Actinomyces* (13%) *Enterobacter* (13%) *Peptoniphilus* (10%) *Gemella* (10%) *Rothia* (10%) *Corynebacterium* (7%) *Bacillus* (7%) *Chrysobacterium* (3%)	Bacteria: 10^6/ml 223 OTUs/sample colostrum (97% sequence similarity) 203 OTUs/sample mature milk	• Bacterial phyla: Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria • High variability in BM microbiota between women (beta diversity) • *S. epidermidis* most frequently detected *Staphylococcus, S. luguduensis, S. hominis, S. microti, S. warneri, S. equorum*), no *S. aureus, S. mitis, S. infantis, S. cristatus, S. salivarius, S. mutans. S. sanguinis, S. gordonii, S. sanguinosus, F. magna, P. deceptionensis, P. fragi, P. meridiana, P. gessardii, P. moorei, P. japonica, P. sasplenii. A. haemolyticus, A. junii, A. ursingii, A. lwoffii, A. parvus, A. guillouiae, A. pitti, P. alcaliphila, Anaerococcus octavius, A. murdochii, A. prevotii, Acitomyces radingae, A. neuii, Enterobacter concerogenus, E. aerogenes, E. hormaechei, E. asburiae, E. kobei, Peptoniphilus lacrimalis, P. gorbachii, P. harei, Gemella hyemolysans* • No differences in number of bacteria over time • No correlation between the number of bacteria and number of immune cells

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Table 1 (*continued*)

Kumar et al.[45] China, Finland, South Africa, Spain 2016	80 (20 each country), 80 33y (range ns) >50% received IAP (cefazolin, penicillin, metronidazole or azithromycin) Probiotic use ns Gestational age ns 50% delivered vaginally Feeding method ns* Cleaning breasts with soap and sterile water, soak in chlorhexidine, manual expression, discarding first drops	16S rRNA gene sequencing InviMag Faeces DNA kit V4 Primers 515F, 806R PCR cycles ns Illumina MiSeq Greengenes Database	1m	*Staphylococcus* *Streptococcus* *Pseudomonas* *Ralstonia* *Acinetobacter* *Phyllobacterium* *Stenotrophomonas* *Rothia* *Corynebacterium*	ns	• Bacterial phyla: Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria • Composition of the BM microbiota differed across countries (higher relative abundance of *Enterobacteriaceae* and *Pseudomonadaceae* in South Africa, Firmicutes in Finland, *Streptococcus* in China, *Cutibacterium* and *Pseudomonas* in Spain). *Lactobacillaceae* was found uniquely in Finland, *Bifidobacteriaceae* in South African and *Enterococcaceae* all countries, but China • Spanish women who delivered by CS had a decreased alpha-diversity and higher relative abundance of Proteobacteria • MUFA were negatively associated with relative abundance Proteobacteria, while *Lactobacillus* genus was positively associated with MUFA
Urbaniak et al.[46] Canada 2016	39, 39 Maternal age ns 10% received AB during pregnancy Probiotic use ns 28% term infants 59% delivered vaginally Feeding method ns* Cleaning breasts with sterile water with sterile gloves, expression by pump	16S rRNA gene sequencing QIAamp DNA faeces mini kit V6 Primers ns 25 PCR cycles Illumina MiSeq Silva Database	6–245d	*Staphylococcus* (31%) *Enterobactericaceae* (10%) *Pseudomonas* (17%) *Streptococcus* (5%) *Lactobacillus* (3%)	Bacteria: 47 genera	• Bacterial phyla: Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes • No difference in the BM microbiota of mother who delivered vaginally or by CS, who had female or male and preterm or term infants and over time

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Table 1 (*continued*)

Sakwinska et al.[47] China 2016	90, 90 28±4y (range ns) No AB during collection Probiotics ns 39.3 ± 1.1 w 49% delivered vaginally 100% exclusively breastfed Cleaning breasts with chlorhexidine with sterile gloves, manual expression, discarding first drops (30) Expression by pump without special cleaning (60)	16S rRNA gene sequencing DNA Faeces Mini kit or DNA SPIN Kit for soil V4 Primers ns 30 PCR cycles Illumina MiSeq Ribosomal Database Project qPCR for total bacterial loads, Bifidobacterium, Lactobacillus,	1–4d 5–11d 1–2m	*Streptococcus* (42%) *Staphylococcus* (40%) *Acinetobacter* *Pseudomonas* *Corynebacterium* *Enterobacteriaceae* *Delftia* *Comamonas* *Gemella* *Enhydrobacter* *Bacillales* *Pasteurellaceae* *Neisseria* *Rothia* *Alcaligneaceae* *Lactococcus* *Veillonella* *Lactobacillus* *Bifidobacterium* *Stenotrophomonas*	Bacteria: PCR: 10^4/ml 10^3/ml for aseptically collected samples	• Higher total bacterial loads in aseptically collected samples with higher relative abundance of *Acinetobacter* • Lactation stage and delivery mode did not influence the composition of the BM microbiota
Dave et al.[48] Mexico 2016	10, 10 25±3y (range ns) Term infants 100% delivered vaginally Feeding method ns* Cleaning hands and breast, expression by pump	16S rRNA gene sequencing QIAamp ultraclean production pathogen mini kit V4 Primers 515 F, 806R 35 PCR cycles Illumina HiSeq Greengenes Database qPCR	2-4d	*Streptococcus* (74%) *Staphylococcus* (11%) *Sediminibacterium* *Prevotella* (0.5%) *Neisseria* (0.1%)	Bacteria: 241 OTUs/sample (average 82) (97% sequence similarity)	
Cabrera-Rubio et al.[49] Spain 2016	10, 10 31±7y (range ns) No antibiotics No probiotics 36.6 ± 4.2 w 60% delivered vaginally 100% exclusively breastfed Cleaning breasts with soap and sterile water, soak in chlorhexidine, manual expression, discarding of first drops	16S rRNA gene sequencing QIAamp DNA faeces mini kit V1-V3 Primers 27F, 533R 20 PCR cycles Genome Sequencer FLX Ribosomal Database Project qPCR	1m	*Pseudomonadaceae* *Streptococcaceae* *Staphylococcaceae* *Enterobacteriaceae* *Leuconostocaceae* *Moraxellaceae* *Lactobacillaceae* *Oxalobacteraceae* *Veillonellaceae* *Cornamonadaceae* *Neisseriaceae* *Ruminococcocaceae* *Lachnospiraceae* *Aeromonadaceae* *Cutibacteriaceae* *Enterococcaceae*	Bacteria: 500 OTUs (vaginally delivered) (97% sequence similarity) 250 OTUs (CS)	• Phyla detected: Proteobacteria (65%), Firmicutes (34%), Bacteroidetes, TM7, Actinobacteria, Fusobacteria • Higher bacterial diversity, lower absolute abundance of *Staphylococcus* and higher absolute abundance of *Streptococcus, Bifidobacterium, Enterococcus* (this was not statistically significant) in BM of women who delivered vaginally

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Table 1 (*continued*)

Bender et al.[67] Haiti 2016	50 (25 HIV-infected), 50 HIV-infected mothers 30±5y 72% antibiotics during pregnancy and post-partum Probiotic use ns 8% preterm infants 76% vaginally delivered 80% exclusively breastfed HIV-uninfected mothers 27±8y 40% antibiotics during pregnancy and post-partum Probiotic use ns 12% preterm infants 96% vaginally delivered 76% exclusively breastfed Collection method ns	16S rRNA gene sequencing PSP Stool Spin kit V4 Primers ns PCR cycles ns Ilumina MiSeq Greengenes Database	<6m	*Streptococcaceae* *Staphylococcaceae* *Corynebacteriaceae* *Micrococcaceae* *Moraxellaceae* *Pseudomonadaceae* *Veillonellaceae* *Prevotellaceae* *Bifidobacteriaceae* *Gemellaceae* *Thermaceae*	ns	• No difference in the composition between HIV-infected and uninfected mothers
Jost et al.[50,51] Switzerland 2013	7, 21 Maternal age ns No IAP Probiotic use ns Term infants 100% delivered vaginally 100% exclusively breastfed Cleaning breasts with aseptic soap, expression by pump	16S rRNA gene sequencing FastDNA SPIN kit for Soil V5-V6 Primers 8F, 1291R 25 PCR cycles Genome Sequencer FLX GenBank Ribosomal Database Project Anaerobic culture	3-6d 9-14d 25-30d	*Pseudomonas* (17%) *Streptococcus* (13%) *Staphylococcus* (9%) *Ralstonia* (8%) *Flavobacterium* (5%) *Cutibacterium* (3%) *Burkholderia* (2%) *Rothia* (<2%) *Bifidobacterium* (<2%) *Corynebacterium* (<2%) *Blautia* (<2%) *Brevunidmonas* (<2%) *Collinsella* (<1%) *Bacteroides* (<1%) *Parabacteroides* (<1%) *Alistipes* (<1%) *Lactobacillus* (<1%) *Clostridium* (<1%) *Coprococcus* (<1%) *Dorea* (<1%) *Faecalibacterium* (<1%) *Oscillibacter* (<1%) *Roseburia* (<1%) *Ruminococcus* (<1%) *Subdoligranulum* (<1%) *Dialister* (<1%) *Veillonella* (<1%) *Escherichia* (<1%)	Bacteria: 193 genera $<10^3$ bacteria/ml 512 OTUs/sample (97% sequence similarity)	• Bacterial phyla: Firmicutes, Actinobacteria, Proteobacteria, Bacteroidetes • *S. epidermidis* (most frequently *Staphylococcus), S. lugdunensis, S. aureus/haeomlyticus/hominis, S. pasteuri/warneri, S. salivarius, S. thermophilus, S. vestibularis, S. mitis/pneumoniae, B. breve, C. ances, P. granulosum, R. mucilaginosa, E. faecalis, E. gallinarum,* L. *brevis,* L. *gasseri, V. atpical/dispar/parvula*

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Cabrera-Rubio et al.[52] Spain 2012	18, 54 32±5y (range ns) 17% IAP, 6% AP during lactation Probiotic use ns Gestational age 40.4 ± 1.1 w 50% vaginally delivered 100% exclusively breastfed Cleaning breasts with iodine swab, manual expression, discarding first drops	16S rRNA gene sequencing QIAamp DNA faeces mini kit V1-V2 Primers 27F, 533R 20 PCR cycles Genome Sequencer FLX Ribosomal Database Project qPCR	<2d 1m 6m	*Weissella* *Leuconostoc* *Staphylococcus* *Streptococcus* *Lactococcus* *Actinetobacter* *Citrobacter* *Veillonella* *Corynebacterium* *Lysinibacillus* *Carnobacterium*	Bacteria: >1000 OTUs/sample (97% sequence similarity)	• *Weisella, Leuconostoc, Staphylococcus, Streptococcus*, and *Lactococcus* predominant in colostrum, *Veillonella, Leptotrichia*, and *Prevotella* (oral microbiota) predominant at 1 and 6 m • BM from obese women less diverse bacterial community, higher absolute abundance of *Lactobacillus* in colostrum and higher total bacterial loads, *Staphylococcus* and lower absolute abundance of *Bifidobacterium* in mature milk • BM from women who delivered by elective CS had a higher absolute abundance of Carnobacteriaceae and a lower absolute abundance of Leuconostocaceae compared with women who delivered vaginally (in colostrum and mature milk)
Hunt et al.[53] USA 2011	16, 47 20–40y (range ns) AB use ns Probiotic use ns Gestational age ns Delivery mode ns Feeding method ns* Cleaning breasts with iodine swab, expression by pump	16S rRNA gene sequencing QIAamp DNA faeces mini kit V1-V2 Primers 27F, 338R 35 PCR cycles Genome Sequencer FLX Ribosomal Database Project	3 samples over 4w	*Staphylococcus* (16%) *Streptococcus* (8%) *Serratia* (8%) *Pseudomonas* (5%) *Corynebacterium* (4%) *Ralstonia* (4%) *Cutibacterium* (4%) *Spingomonas* (2%) Other (53%)	Bacteria: 100–600 OTUs/sample (97% sequence similarity)	• Community stable within one individual over time
Solis et al.[54] Spain 2010	20, 80 Maternal age ns 25% IAP (ampicillin 5) Probiotic use ns Term infants (39.2, 95% CI; 38.6–39.7) 100% delivered vaginally 100% exclusively breastfed Cleaning breasts with sterile swab, manual expression or by pump, discarding first drops	16S rRNA gene sequencing GenElute™ Bacterial Genomic DNA Kit V1-V2 Primer ns PCR cycles ns Seqeuencing machine ns GenBank	1d 10d 30d 90d	*Streptococcus* (36–65%) *Staphylococcus* (29–50%) *Lactobacillus* (5%) *Bifidobacterium* (5%)	Bacteria: 10^5 CFU/ml on d1, 10^4 CFU/ml on d90 *Bifidobacterium* 10^3–10^5 CFU/ml on d90	• *S. epidermidis* the most frequently detected *Staphylococcus* • L. *gasseri* the most frequently detected *Lactobacillus* • *B. longum* and *B. breve* the most frequently detected *Bifidobacterium* (others *B. bifidum, B. pseudocatenulatum)* • *E. faecalis* the most frequently detected *Enterococcus* • *S. salivarius* the most frequently detected *Streptococcus /S. vestibularis)* • Women who received IAP had lower total bacterial loads (10^4 vs. 10^6 CFU/ml)

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Martin et al.[55] Spain 2006	10, 10 Maternal age ns AB use ns Proiotic use ns Term infants 50% delivered vaginally Feeding method ns* Collection method ns	16S rRNA gene sequencing Nucleo Spin Extract II kit V6-V8 Primers 8F, 1510R 35 PCR cycles Sequencing machine ns GenBank and Ribosomal Database Project PCR-DGGE	7d	*Streptococcus* *Staphylococcus* *Enterococcus* *Weissella* *Leuconostoc* *Lactococcus* *Lactobacillus* *Cutibacterium* *Escherichia* *Serratia* *Acinetobacter* *Veillonella* *Gemella* *Pseudomonas*	ns	• *S. epidemidis* the most frequently detected *Staphylococcus* • *S. mitis* the most frequently detected *Streptococcus* • *Leuconostoc citreum* and *Lactococcus lactis* the most frequently detected lactic acid bacteria
PCR, PCR-DGGE						
Huang et al.[56] Taiwan 2019	30, 30 29y (17–43) 73% received AB during collection (cefazolin 19, cephalexin 1, amoxicillin 1, cefazolin and amoxicillin 1) Probiotic use ns Gestational age ns 37% delivered vaginally Feeding method ns* Clean breasts with water, manual expression with sterile gloves, discarding 1–2 ml	PCR DNA extraction method ns Culture	ns	*Streptococcus* *Staphylococcus* *Cutibacterium* *Enterococcus* *Acinetobacter* *Enterobacter* *Rothia* *Micrococcus* *Pseudomonas* *Moraxella* *Enhydrobacter* *Corynebacterium*	Bacteria: 40/ml – $10^{7.1}$/ml	
Aakko et al.[57] Finland 2017	11, 11 Maternal age No perinatal AB 64% received probiotics (*B. lactis* ±.L. *rhamnosus GG (LGG)*) 38.7 ± 0.8 w (37.4–39.3) 0% delivered vaginally Feeding method ns* Cleaning breasts with sterile water, soaked in chlorhexidine, manual expression with sterile gloves, discarding first drops	qPCR for *Bacteroides, Bifidobacterium, Clostridium* cluster IV, *Clostridium* cluster XIVa-XIVb, *Enterococcus, Lactobacillus, Staphylococcus, Streptococcus* QIAamp DNA faeces mini kit	1d	*Bifidobacterium* (100% of samples, $10^{4.6}$ copies/g) *Staphylococcus* (100%, $10^{4.5}$) *Streptococcus* (100%, 10^{2}) *Lactobacillus* (100%, $10^{3.4}$) Bacteroides-Prevotella (100%, $10^{2.8}$) *Enterococcus* (82%, $10^{1.7}$) *Akkermansia* (64%, $10^{\circ.9}$) *Clostridium* cluster XIVa-XIVb (82%, $10^{4.6}$) *Clostridium* cluster IV (82%, $10^{2.8}$)	Bacteria: $10^{5.1}$ copies/g	*B. longum* (100% of all samples), *B. breve* (46%), *B. bifidum* (100%), *S. aureus* (46%), *A. muciniphila* (64%) HMO concentration positively associated with absolute abundance of *Bifidobacterium*, sialylated HMO with *B. breve*, non-fucosylated/non-sialylated HMO with *B. longum*, fucosylated HMO with *A. muciniphila* and fucosylated/silylated HMO with *S. aureus*

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Obermajer et al.[58] Slovenia 2015	45, 45 Maternal age ns AB use ns Probiotic use ns Gestational age ns Delivery mode ns Feeding method ns* Cleaning hands and breasts with soap and sterile water, manual expression, discarding first drops	PCR-DGGE qPCR for *Staphylococcus, Streptococcus, Bacteroides-Prevotella, Clostridia, Enterobacteria, Bifidobacterium, Lactobacillus, Enterococcus* Maxwell 16 Tissue DNA Purification K	2-3d	*Staphylococcus* (97% of samples, median 10^3/ml) *Streptococcus* (62%, 10^3/ml) *Clostridium* cluster XIV (96%, 10^4/ml) *Clostridium* cluster IV (64%, 10^3/ml) *Enterobacteriaceae* (100%, 10^3/ml) *Bifidobacterium* (53%, 10^3/ml) *Bacteroides-Prevotella* (62%, 10^3/ml) *Enterococcus* (9%, 10^3/ml) *Lactobacillus* (0%)	Bacteria: 10^8/ml	*S. epidermidis* most prevalent (61% of all Staphylococci)
Khodayar-Pardo et al.[59] Spain 2014	32, 96 23–35y (range ns) 28% AB before delivery, 41% during delivery, no AB during lactation No probiotic use 59% term infants, 27–40 w 47% delivered vaginally 100% exclusively breastfed Cleaning hands with soap, cleaning breasts with chlorhexidine, expression by pump	qPCR for total bacterial loads, *Bifidobacterium, Lactobacillus, Enterococcus, Staphylococcus, Streptococcus* QIAamp DNA faeces mini kit	1-5d 15-16d 16-20d	Colostrum *Enterococcus* (100% of samples, $10^{3.7}$/ml) *Lactobacillus* (78%, $10^{4.2}$/ml) *Streptococcus* (72%, $10^{3.7}$/ml) *Bifidobacterium* (65%, $10^{1.9}$/ml) *Staphylococcus* (47%, $10^{3.1}$/ml) Mature milk *Lactobacillus* (100% of samples, $10^{4.5}$/ml) *Enterococcus* (100%, 10^4/ml) *Staphylococcus* (100%, $10^{3.6}$/ml) *Bifidobacterium* (100%, $10^{2.1}$/ml) *Streptococcus* (78%,$10^{3.6}$/ml)	Bacteria: colostrum $10^{4.5}$/ml, mature milk $10^{5.2}$/ml	• Total bacterial loads and relative and absolute abundance of *Bifidobacterium* and *Enterococcus* increased throughout lactation period • BM of women with term infants had higher relative and absolute abundance of *Bifidobacterium* • Total bacterial number was higher in BM of women CS • *Bifidobacterium* was detected more frequently in women who delivered vaginally

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Gonzalez et al.[60] Mozambique 2013	144, 55 Maternal age ns AB use ns Probiotic us ns 24% HIV positive Gestational age ns Delivery mode ns Feeding method ns* Breasts not cleaned or sterilised manual expression, discarding first 2 drops	qPCR QIAamp DNA faeces mini kit Culture	ns	*Staphylococcus* (96% of samples) *Streptococcus* (93%) *Lactobacillus* (56%) *Kocuri* (31%) *Rothia* (24%) *Gemella* (22%) *Bifidobacterium* (11%)	ns	• *S. epidermidis* ($10^{3.4}$/ml), *S. hominis* ($10^{2.8}$/ml), *S. aureus* (10^{1}/ml) • *S. salivarius* ($10^{1.7}$/ml), *S. mitis* ($10^{1.1}$/ml), *S. parasanguis* ($10^{1.2}$/ml) • L. *gastricus* ($10^{0.8}$/ml), L. *fermentum* ($10^{0.3}$/ml), L. *gasseri* ($10^{0.3}$/ml) • Women who exclusively breastfed had a higher proportion of *S. parasanguis* compared with women who used mixed infant feeding • Women who were HIV positive had higher absolute abundance of *Lactobacillus*
Collado et al.[61] Spain 2009	50, 50 Maternal age ns AB use ns Probiotic use ns Term infants Delivery mode ns Feeding method ns* Cleaning breasts with sterile water, soaked in chlorhexidine, manual expression with sterile gloves, discarding first drops	qPCR for *Bacteroides, Bifidobacterium, Clostridium* cluster IV, *Clostridium* cluster XIVa-XIVb, *Enterococcus, Lactobacillus, Staphylococcus, Streptococcus* QIAamp DNA faeces mini kit	ns	*Staphylococcus* (100% of samples; mean 10^{4}/ml) *Streptococcus* (100%; 10^{5}/ml) *Bifidobacterium* (100%; 10^{4}/ml) *Lactobacillus* (100%; 10^{4}/ml) *Clostridium* XIVa-XIVb (96%; 10^{3}/ml) *Enterococcus* (76%; 10^{3}/ml) *Bacteroides* (40%; 10^{2}/ml) *Clostridium* IV (4%; 10^{2}/ml)	Bacteria: 10^{6}/ml	
Culture						
Chen et al.[62] Taiwan 2016	19, 19 Maternal age ns AB use ns Probiotic use ns Gestational age ns Delivery mode ns Feeding method ns* Cleaning of breasts with water, manual expression with sterile gloves, discarding 0.5 ml	Culture	3-360d	*Staphylococcus* *Streptococcus* *Enterococcus* *Lactobacillus* *Kluyvera* *Klebsiella* *Acinetobacter* *Actinomyces*		• *S. epidermidis, S. lugdunensis, S. haemolyticus, S. aureus, S. pasteuri, S. mitis, S. pneumoniae, S. parasanguinis, E. faecalis, E. malodoratus, A. baumannii, K. ascorbate, K. pneumoniae, K. oxytoca, A. bovis,* L. *gasseri*

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Moles et al.[63] Spain 2015	22, 51 Maternal age ns 68% received AB Probiotic use ns 24–27 w 36% delivered vaginally Feeding method ns* Breasts not cleaned or sterilised manual expression	Culture	1-4d 14-56d	Colostrum *Staphylococcus* (35% of samples, $10^{3.3}$/ml) *Streptococcus* (18%, 10^{2}/ml) *Lactobacillus* (12%, $10^{2.8}$/ml) *Enterobacteria* (6%, $10^{2.6}$/ml) Mature milk *Enterococcus* (29%, $10^{2.8}$/ml) *Staphylococcus* (23%, $10^{2.7}$/ml) *Lactobacillus* (23%, $10^{4.2}$/ml) *Enterobacteria* (15%, 10^{3}/ml) *Streptococcus* (12%, $10^{3.1}$/ml)	Bacteria: Colostrum 10^{2}–$10^{3.3}$/ml Mature milk 10^{2}–$10^{4.2}$/ml	• Mature milk contained higher absolute abundance of *Enterococcus, Lactobacillus* and *Streptococcus*
Dahaban et al.[64] Malaysia 2013	35, 35 Maternal age ns AB use ns Probiotic us ns 31 w (range ns) Delivery method ns Feeding method ns* Collection mode ns	Culture	ns	*Acinetobacter* (40% of samples) *Klebsiella* (37%) *Bacillus* (17%) *Staphylococcus* (14%) *Enterobacter* (14%) *Escherichia* (6%) *Pseudomonas* (3%) *Micrococcus* (3%)	Bacteria: 40% > 10^{4}/ml	• Women who delivered by CS had lower total bacterial loads • Higher total bacterial loads after first week of life
Jiménez et al.[65] Spain 2008	16, 48 Maternal age ns Antibiotic use ns Probiotic use ns Term infants 100% vaginally delivered Feeding method ns* Collection method ns	Culture	7d 14d 35d	*Staphylococcus* (67%) *Escherichia* (8%) *Streptococcus* (7%) *Enterococcus* (4%) *Enterobacter* (1%) *Klebsiella* (1%) *Bifidobacterium* (1%) *Acinetobacter* (1%) *Citrobacter* (1%) *Lactobacillus* (0.4%) *Burkholderia* (0.4%)		• *S. epidermidis* (100% of samples), *S. aureus* (16%), • *E. faecalis* (21%) • *B. adolescentis, B. brevis, B. infantis, B. bifidum, B. longum, B. seudocatenulatum, B. dentium, B. angulatum*

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Table 1 (*continued*)

Heikkila et al.[66] Finland 2003	40, 40 Maternal age ns AB use ns Probiotic use ns Gestational age ns Delivery mode ns Feeding method ns* Cleaning breasts with water, manual expression or by pump	Culture Identification with RFLP or partial sequencing of 16S rRNA gene	< 3m (75%) 3–14m (25%)	*Staphylococcus* (64%; 98% of samples) *Streptococcus* (30%; 73%) LAB (13% of samples) *Enterococcus* (4% of samples)	Bacteria: 10^3 - 10^4 bacteria/ml 10–21 isolates/sample	• *S. epidermidis* most frequently detected *Staphylococci* (98% of samples), other *Staphylococci* were *S. hominis, S. capitis, S. lugdunensis, S. aureus* (13%) • *S. salivarius* (45% of samples) and *S. mitis* (28%) most frequently detected *Streptococci*, other *Streptococci* were *S. peroris, S. oralis* • *L crispatus, L rhamnosus,* L. *lactis,* L. *mesenteroides* • *E. faecalis*

AB, antibiotic use; BM, breast milk; BMI, body-mass index; CS, Caesarian section; d, day; DGGE, denaturing gradient gel electrophoresis; HMO, human milk oligosaccharides; IAP, intrapartum antibiotics; ns, not specified; m, month; MALDI-TOF-MS, Matrix Assisted Laser Desorption Ionization-Time of Flight mass spectrometry; MUFA, Monounsaturated fatty acids; OTUs, operational taxonomic units; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; w, week.

* % of exclusively breastfed infants.

Table 2
Summary of methods in studies which used 16S rRNA gene sequencing for assessing the breast milk microbiota.

Reference	DNA extraction kit	Hypervariable region	Primers	Sequencing machine	Database
Albesharat et al.[29]	E.Z.N.A. bacterial DNA kit	ns	609F, 616R	ns	ns
Hermansson et al.[30]	InviMag Faeces DNA kit	V3-V4	515F, 806R	Illumina MiSeq	Greengenes Database
Ojo-Okunola et al.[31]	ZR Fungal/Bacterial DNA MiniprepTM	V4	515F, 806R	Illumina MiSeq	Ribosomal Database Project
Ding et al.[32]	Ezup Column Bacteria Genomic DNA Purification kit	V3-V4	ns	Illumina MiSeq	Ribosomal Database Project
Moossavi et al.[33]	Quick-DNA Fungal/Bacterial extraction kit	V3-V4	515F, 806R	Illumina MiSeq	Greengenes Database
Simpson et al.[34]	LGC Mag DNA extraction kit	V3-V4	Uiv_F, Univ_r	Illumina MiSeq	Ribosomal Database Project
Tuominen et al.[35]	High-salt method	V3-V4	ns	Illumina MiSeq	Greengenes Database
Li et al.[36]	QIAquick PCR purification kit	V1-V2	27F, 228R	Genome Sequencer FLX	Greengenes Database
Toscano et al.[37]	Milk DNA extraction kit	V2-4-8 and V3-6, 7-9	ns	Ion Torrent Personal Genome Machine	Greengenes Database
Pannaraj et al.[38]	BiOstic Bacteremia DNA isolation kit	V4	27F, 228R	Illumina MiSeq	Greengenes Database
Williams et al.[39]	QIAamp DNA faeces mini kit	V1-V3	27F, 534R	Illumina MiSeq	Ribosomal Database Project
Biagi et al.[40]	DNeasy Blood&Tissue kit	V3-V4	S-D-Bact-0341-b-S-17, S-D-Bact-0785-a-A-21	Illumina MiSeq	Greengenes Database
Drago et al.[41]	Milk DNA extraction kit	V2-4-8 and V3-6, 7-9	ns	Ion Torrent Personal Genome Machine	Greengenes Database
Cacho et al.[42]	PowerFecal® DNA isolation kit	V4	515F, 806R	Illumina HiSeq	Greengenes Database
Murphy *et al*[19]	QIAamp DNA faeces mini kit	V3-V4	ns	Illumina MiSeq	BLAST database
Patel et al.[43]	QIAamp fast DNA faeces mini kit	V2-V3	101F, 518R	Ion Torrent Personal Genome Machine	Greengenes Database
Boix-Amoros et al.[44]	MasterPure complete DNA and RNA purification kit	ns	8F, 785R	Genome Sequencer FLX	Ribosomal Database Project
Kumar et al.[45]	InviMag Faeces DNA kit	V4	515F, 806R	Illumina MiSeq	Greengenes Database
Urbaniak et al.[46]	QIAamp DNA faeces kit	V6	ns	Illumina MiSeq	SILVA Database
Sakwinska et al.[47]	DNA Faeces Mini kit or DNA SPIN Kit for soil	V4	ns	Illumina MiSeq	Ribosomal Database Project
Dave et al.[48]	QIAamp ultraclean production pathogen mini kit	V4	515 F, 806R	Illumina HiSeq	Greengenes Database
Cabrera-Rubio et al.[49]	QIAamp DNA faeces mini kit	V1-V3	27F, 533R	Genome Sequencer FLX	Ribosomal Database Project
Bender et al.[67]	PSP Stool Spin kit	V4	Illumina MiSeq	Greengenes Database	
Jost et al.[50,51]	FastDNA SPIN kit for Soil	V5-V6	8F, 1291R	Genome Sequencer FLX	GenBank and Ribosomal Database Project
Cabrera-Rubio et al.[52]	QIAamp DNA faeces mini Kit	V1-V2	27F, 533R	Genome Sequencer FLX	Ribosomal Database Project
Hunt et al.[53]	QIAamp DNA faeces mini Kit	V1-V2	27F, 338R	Genome Sequencer FLX	Ribosomal Database Project
Solis et al.[54]	GenEluteTM Bacterial Genomic DNA Kit	V1-V2	ns	ns	GenBank
Martin et al.[55]	Nucleo Spin Extract II kit	V6-8	8F, 1510R	ns	GenBank and Ribosomal Database Project

ns, not specified.

11, GenBank 3, BLAST Database 1, Silva Database 1, not stated (ns) 1) were used.

General composition

Bacteria

Four studies (10, 36, 7 and ns participants respectively) reported that bacterial diversity of the microbiota is higher in BM compared with infant or maternal faeces.[19,40,51,88] The maximum number of each bacterial taxonomic level detected per study was 58 phyla,[31] 133 classes,[31] 263 orders,[31] 596 families,[31] 590 genera,[43] 1300 species[31] and 3563 operational taxonomic units (OTUs), defined as 97% sequence similarity).[36] Between 22 to 260 species[27,36] and 203 to 512 OTUs[44,48,50,51] were detected per BM sample. Culture-based methods reported median bacterial loads between $10^{1.5}$ and 10^{6} CFU/ml[26,27,36,54,55,59,63,64,66,88] and studies using qPCR between 10^{4} and 10^{8}/ml.[44,47,58,59,61] With an average intake of 800 ml of BM a day, an infant ingests approximately 8×10^{7} to 10^{10} bacteria per day. One small study (21 participants) reported that bacterial diversity and richness does not correlate with the total number of bacteria.[44]

Most studies reported Firmicutes and Proteobacteria to be the most predominant phyla in BM, while Actinobacteria and Bacteroidetes were present at lower relative abundances.[19,28,30,33–35,38,43,46,49] Two studies reported Firmicutes to be the most abundant phyla, followed by Actinobacteria and then Protebacteria.[31,39] Further phyla detected in BM were Acidobacteria Armatimonadetes, Chlamydiae, Chlorobi, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Elusimicrobioa, Fibrobacteres, Fusobacteria, Planctomycetes, Spirochaetes, Synergistetes, Tenericutes, Thermi, Sacharibacteria, Verrucomicrobia and WPS2.[27,30,31,43,49,50]

Although up to 590 different genera have been isolated from BM,[43] a core microbiota of 7–9 bacterial genera was often found.[19,31,36,41,44,53] The 55 genera which were isolated from more than two studies are summarised in Table 3. Thirty-eight of the 43 included studies reported results at the genus level. The most frequently found genera were: *Staphylococcus* (genera found in 97% of studies; range of relative abundance 5–83%), *Streptococcus* (95%; <1 to 74%), *Lactobacillus* (63%; <1 to 5%), *Pseudomonas* (50%; <1 to 17%), *Bifidobacterium* (42%; <1 to 5%), *Corynebacterium* (42%; <1 to 6%), *Enterococcus* (42%; 1%), *Acinetobacter* (39%; 2 to 4%), *Rothia* (34%; 1 to 6%), *Cutibacterium* (29%; <1 to 3%), *Veillonella* (24%; <1 to 6%), *Bacteroides* (24%; <1 to 4%), *Gemella* (21%; 0.7 to 13%), *Prevotella* (21%; <1 to 9%), *Klebsiella* (16%), *Clostridium* (16%; <1%), *Stenotrophomonas* (16%; 2 to 3%), *Enterobacter* (13%; 1%), *Escherichia* (13%; <1 to 8%), *Actinomyces* (13%, <1 to 1%), *Neisseria* (13%; <1 to 2%), *Serratia* (13%; 2 to 8%), *Burkholderia* (13%; <1 to 2%), *Delftia* (11%; <1%), *Micrococcus* (11%; <1%), *Spingomonas* (11%; <1 to 2%), *Haemophilus* (11%; <1 to 4%), *Halomonas* (11%; 1to 26%), *Lactococcus* (11%), *Pantoea* (11%; 1%), *Ruminococcus* (11%; <1%). The remaining genera were found in less than 10% of studies. One study (133 participants) reported that the abundance of Gram positive bacteria (*Staphylococcus, Streptococcus* and *Rothia*) was negatively correlated with the relative abundance of Gram negative bacteria (*Acinetobacter, Bacteroides, Halomonas, Herbaspirillum* and *Pseudomonas*).[36] The number of species isolated per study varied from 6 to 1300 (detailed in Table 1).[26,27,31,32,36,44,45,52,53]

Three studies (107, 16 and 21 participants respectively) reported, that the composition of the BM microbiota remains stable over time in an individual.[38,44,53] However, three studies (20, 21 and 90 participants respectively) described a high variability in the composition of the BM microbiota between women (beta-diversity).[27,44,47]

Microbes other than bacteria

There was only one small study (20 participants) that investigated the presence of microbes other than bacteria.[27] The study used shotgun sequencing. Fungal DNA was detected in 85% (17/20) of samples with an average of 1 to 5 fungal species/sample. The detected fungi belonged to 2 phyla (*Basidiomycota* and *Ascomycota*) and 10 species (*Malassezia globosa* (the most frequently detected), *Calocera cornea, Guepiniopsis buccina, Podospora anserine, Sordaria macrospora, Candida dubliniensis, M. restricta, Talaromyces stipitatus,* and *Yarrowia lipolytica*). Archaeal DNA was detected in 80% (8/10) of samples from healthy women (*Haloarcula marismortui, H. utahensis* and *H. muko*), but none of the women with mastitis. Eukaryotic DNA was detected in all samples (20/20). *Toxoplasma gondii* was detected in 35% (7/20) and *Giardia lamblia* in one sample. The other eukaryotes were *Dicyostelium discoideum, D. purpureum* and *Paramecium tetraurelia.* Viral DNA was detected in 100% of the samples and the sequences were related to *Papillomaviridae, Retroviridae, Siphoviridae* and *Herpesviridae* (*Betapapillomavirus, Cytomegalovirus, Lentivirus, Simplexvirus, Staphylococcus* phage, human endogenous retroviruses).

Factors that influence the breastmilk microbiota

Gestational age

The macronutrients and immunological components of BM of women who deliver at term or preterm differ.[89] Two studies investigated the influence of gestational age on the BM microbiota.[46,59] One study (32 participants) reported a higher relative and absolute abundance of *Bifidobacterium* in women who delivered at term,[59] while another study (39 participants) reported no effect of gestational age on composition.[46]

Delivery mode

Delivery mode has been reported to affect macronutrient concentrations in BM.[90] In relation to the microbiota composition of BM, it has been suggested that labour might lead to an increased intestinal permeability, enhanced bacterial translocation in the maternal gut and consequently a higher transfer of bacteria into BM.[52] In line with this, five studies (84, 29, 20, 393, 10 participants respectively) reported a higher alpha-diversity in BM of women who had delivered vaginally.[30,33,37,45,49] One study (133 participants) did not confirm this.[36] Total bacterial loads have been reported to be higher in BM of women who had delivered by CS (32 participants) in one study,[59] but lower in another study (35 participants).[64] Furthermore, women who delivered by CS have been observed to have higher relative abundance of environmental bacteria in BM (29 participants).[37]

Women who delivered by CS have been reported to have a higher relative abundance of Proteobacteria (20 participants),[45] *Carnobacteriaceae* (18 participants),[52] *Lactobacillus* (133 participants),[36] and unclassified OTUs (133 participants),[36] but a lower relative abundance of *Leuconostocaceae* (18 participants)[52] compared with women who had delivered vaginally. Vaginal delivery, on the other hand, has been associated with higher relative abundance of *Bifidobacterium* (10, 32 participants),[49,59] *Haemophilus* (29 participants),[37] *Streptococcus* (89, 29, 10 participants respectively),[32,37,49] *Lactobacillus* and lower relative abundance of *Finegoldia* (29 participants),[37] *Halomonas* (29 participants),[37] *Staphylococcus* (89, 29, 10 participants respectively),[32,37,49] *Prevotella* (29 participants)[37] and *Pseudomonas* (29 participants).[37] One study (47 participants) found *L. gasseri* only in women who had delivered vaginally.[26] Three studies (21, 393, 39 participants

Table 3
Summary of genera isolated from breast milk (only those isolated in more than two studies included). The last row depicts the total number of studies from which each genera has been isolated).

Aerobic

Reference	*Achromobacter*	*Acinetobacter*	*Citrobacter*	*Chryseobacterium*	*Delftia*	*Enterobacter*	*Enterococcus*	*Escherichia*	*Klebsiella*	*Lysinibacillus*	*Methylobacterium*	*Micrococcus*	*Sphingobium*	*Spingomonas*
25								✓						
26														
27							✓				✓		✓	✓
28														
29							✓							
31		✓										✓		
32	✓	✓					✓						✓	
33														
34		✓							✓		✓			
35					✓					✓				✓
36					✓							✓		✓
37														
39														
41	✓	✓			✓		✓		✓					
42		✓		✓			✓							
19				✓		✓								
43		✓					✓		✓		✓			
44		✓		✓		✓								
45		✓												
46														
47		✓			✓									
48														
51								✓						
52		✓	✓							✓				
53														✓
54														
55		✓					✓	✓						
56		✓				✓	✓					✓		
57							✓							
58							✓							
59							✓							
60														
61							✓							
62		✓					✓		✓	✓				
63							✓							
64		✓				✓		✓	✓			✓		
65		✓	✓			✓	✓	✓	✓					
66							✓							
	2	15	2	3	4	5	16	5	6	3	3	4	2	4

Facultative anaerobic

Reference	*Actinomyces*	*Aeromonas*	*Agrobacterium*	*Corynebacterium*	*Cutibacterium*	*Edwardsiella*	*Enhydrobacter*	*Flavobacterium*	*Gemella*	*Granulicatella*	*Haemophilus*	*Halomonas*	*Lactobacillus*	*Lactococcus*	*Neisseria*	*Pantoea*	*Pseudomonas*	*Ralstonia*	*Rothia*	*Serratia*	*Staphylococcus*	*Stenotrophomonas*	*Streptococcus*	*Weissella*
25													✓						✓		✓		✓	
26	✓			✓									✓								✓		✓	
27				✓	✓								✓		✓		✓				✓		✓	
28						✓										✓	✓				✓		✓	
29													✓										✓	
31				✓					✓										✓		✓		✓	
32		✓			✓				✓				✓	✓		✓					✓		✓	
33																		✓			✓		✓	
34										✓	✓		✓						✓		✓	✓	✓	
35	✓		✓	✓	✓						✓		✓		✓		✓		✓		✓		✓	
36				✓	✓							✓	✓				✓		✓		✓	✓	✓	
37											✓	✓					✓				✓		✓	
39	✓			✓	✓				✓	✓	✓		✓		✓		✓		✓		✓		✓	
41				✓	✓			✓	✓			✓				✓	✓		✓	✓	✓		✓	
42			✓	✓								✓	✓				✓			✓	✓	✓	✓	
19								✓					✓				✓				✓	✓	✓	
43		✓		✓	✓	✓							✓			✓	✓	✓		✓	✓			
44	✓			✓					✓								✓		✓		✓		✓	
45				✓													✓	✓	✓		✓	✓	✓	
46													✓				✓				✓		✓	
47				✓			✓		✓				✓	✓	✓		✓		✓		✓	✓	✓	
48															✓						✓		✓	
51				✓	✓			✓					✓				✓	✓	✓		✓		✓	
52				✓										✓							✓		✓	✓
53				✓	✓												✓	✓		✓	✓		✓	
54													✓								✓		✓	
55					✓				✓				✓	✓			✓			✓	✓		✓	✓
56				✓	✓		✓										✓		✓		✓		✓	
57													✓								✓		✓	
58													✓								✓		✓	
59													✓								✓		✓	
60									✓				✓						✓		✓		✓	
61													✓								✓		✓	
62	✓																				✓		✓	
63													✓								✓		✓	
64																	✓				✓			
65													✓								✓		✓	
66																					✓		✓	
	5	2	2	16	11	2	2	3	8	2	4	4	24	4	5	4	19	5	13	5	37	6	36	2

Anaerobic

Reference	*Akkermansia*	*Bacillus*	*Bacteroides*	*Bifidobacterium*	*Blautia*	*Burkholderia*	*Clostridium*	*Eubacterium*	*Faecalibacterium*	*Finegoldia*	*Leuconostoc*	*Parabacteroides*	*Prevotella*	*Roseburia*	*Ruminococcus*	*Subdoligranulum*	*Veillonella*
25	✓		✓	✓	✓			✓							✓	✓	
26				✓													
27			✓	✓		✓		✓	✓			✓		✓	✓		
28																	
29																	
31																	✓
32		✓		✓						✓							
33																	
34																	✓
35						✓							✓				✓
36			✓	✓													✓
37										✓			✓				
39				✓			✓						✓				✓
41			✓								✓		✓				
42		✓	✓														
19				✓													
43		✓				✓	✓	✓	✓				✓		✓		✓
44		✓								✓							
45																	
46																	
47				✓													✓
48													✓				
51			✓	✓	✓	✓	✓		✓			✓		✓	✓	✓	✓
52											✓						✓
53																	
54				✓													
55											✓						
56																	
57	✓		✓	✓			✓						✓				
58			✓	✓			✓						✓				
59				✓													
60				✓													
61			✓	✓			✓										
62																	
63																	
64		✓															
65				✓		✓											
66																	
	2	5	9	16	2	5	6	3	3	3	3	2	8	2	4	2	9

respectively) found no association between the composition of the BM microbiota and delivery mode.[33,39,46]

Biological sex

Biological sex has been described to influence the composition of BM: women who give birth and then nurse male infants have been reported to have higher calorie contents in BM.[91] Three studies have investigated the influence of biological sex on the composition of the BM microbiota.[33,43,46] One study (393 participants) reported a lower diversity and richness in the BM microbiota of mothers of male infants[33] and another (21 participants) reported that mothers of male infants had a higher relative abundance of *Streptococcus* and a lower relative abundance of *Staphylococcus* in their BM.[39] In contrast, a third study (39 participants) did not find an association between biological sex and the BM microbiota.[46]

Parity

One large study (393 participants) reported a lower diversity in the BM microbiota in infants who were first born compared with these who had one or more sibling.[33] However, two other studies did not find an effect of parity on the composition of the BM microbiota (21, 393 participants respectively).[33,39]

Intrapartum antibiotics

Women who received intrapartum antibiotics (IAP) have been reported to have lower total bacterial loads (10^4 vs. 10^6 CFU/ml) (20 participants),[54] but a higher alpha-diversity and richness in their BM microbiota.[30] One study (84 participants) reported that *Bifidobacterium* could not be found in women who had received IAP.[30] Similarly, two other studies, which were not included in this review because the overall composition of the BM microbiota was not investigated, showed that women who received IAP had

a lower relative and absolute abundance of *Bifidobacterium* and *Lactobacillus,* while no effect of IAP on total bacterial loads was observed.[73,84]

Antibiotic resistance

Only one small study (16 participants) investigated antibiotic resistance in the BM microbiota.[25] Shotgun sequencing showed that there was an overlap of mobile genetic elements in BM and infant faeces.[25] This adds to the evidence that antibiotic resistance can be transferred from mothers to their infants.[92]

Human immunodeficiency virus (HIV) infection

Two studies compared the BM of women infected with human immunodeficiency virus (HIV) and non-HIV infected women.[60,67] One study (144 participants) found the former had a higher relative and absolute abundance of *Lactobacillus* in their BM,[60] Another study compared the composition of the BM microbiota between HIV-infected and HIV-uninfected mothers and did not find a difference.[67] However, all HIV-infected mothers were on antiretroviral therapy with low levels of HIV RNA (68% undetectable) and high CD4 T cells counts.

Lactation stage

The composition of BM changes during lactation: colostrum, which is generally produced until the fifth or sixth day after delivery, is rich in proteins and minerals, and contains many immune active substances, such as antibodies, complement factors, cytokines, lysozyme, oligosaccharides and antimicrobial peptides.[93,94] One month after birth, BM achieves is mature composition and contains lower concentrations of proteins and minerals, and higher concentrations of lipids and carbohydrates.[94]

Several studies have investigated the composition of the BM microbiota over time.[19,26,34,36,38,39,44,46,47,52–54,59,63,64] Two studies (47, 80 participants respectively) reported higher total bacterial loads in colostrum compared with mature milk.[26,54] In contrast, two other studies (32, 35 participants respectively) reported an increase in total bacterial loads throughout the lactation period.[59,64] One study (21) did not show changes in the number of bacteria in BM during the first month of life.[44] Additionally, one study (125) reported a higher diversity at 3 months of age compared with 10 days,[34] and another small study (10) reported an increase in bacterial richness from 3 to 6 week and decrease in richness and diversity from 6 to 12 weeks.[19]

Two studies (21, 20 participants respectively) reported changes in composition of BM over time without a clear pattern.[44,54] Furthermore, higher relative abundances of *Enterococcus* (18 participants),[63] *Lactococcus* (18 participants),[52] *Leuconostoc* (18 participants),[52] *Staphylococcus* (125, 18 participants respectively),[34,52] *Streptococcus* (18, 22 participants respectively)[52,63] and *Weissella* (18 participants)[52] have been reported during the first 10 days of life, while *Bifidobacterium* (32 participants),[59] *Granulicatella* (125, 21 participants respectively),[34,39] *Lactobacillus* (22, 32 participants respectively),[59,63] *Leptotrichia* (18 participants),[52] *Methylobacterium* (125 participants),[34] *Prevotella* (18 participants),[52] *Rothia* (125 participants),[34] and *Veillonella* (125, 21, 18 participants respectively)[34,39,52] have been reported to be more abundant thereafter. On study (50 participants) reported a greater relative abundance of anaerobic bacteria in mature milk compared with colostrum.[41] In three studies (133, 39, 90 participants respectively), the lactation stage was not observed not influence the composition of the BM microbiota.[36,46,47] However, these studies did not specifically investigate colostrum.

Diet

Only few studies have investigated the influence of diet on the composition of the BM microbiota.[29,39,41] One study (50 participants) found no association between diet and the BM microbiota.[41] Another study (21 participants) found that calorie intake was positively associated with the relative abundance of *Granulicatella*.[39] In Syria, *L. plantarum* was found as a major component of the BM microbiota. It was suggested that this comes from food, as it is commonly detected on fermented vegetables which are frequently consumed in Syria.[29] Similarly, *Rhizobium* as a symbiont on legumes, which are the main component of the diet in women in Burundi, was one of the main bacteria found in BM in Burundi.[41] One small study (21 participants), reported that a higher intake of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) was associated with a lower relative abundance of *Corynebacterium* in BM.[39]

Body mass index

Women with high body mass indexes (BMIs) have been reported to have a less diverse bacterial community in the BM microbiota with higher total bacterial loads and a higher absolute abundance *Lactobacillus* in colostrum (18 participants).[52] Furthermore, women with a high BMI have been observed to have a higher relative abundance of *Akkermansia* (18 participants),[52,95] *Granulicatella* (21 participants)[39] and *Staphylococcus* (89 participants)[32] and a lower relative abundance of *Bacteorides* (21 participants),[39] *Bifidobacterium* (18 participants),[52] *Lactobacillus* (89 participants)[32] and *Streptococcus* (89 participants)[32] in mature milk. One study (47 participants) only detected *L. gasseri* in women with normal weight.[26] Two studies (133, 393 participants respectively) did not find any influence of BMI on the composition of the BM microbiota.[33,36]

Macro- and micronutrients and bioactive substances

One study (21 participants) showed the concentration of lactose in BM was negatively correlated with the abundance of *Enterobacter* and *Actinomyces,* and concentrations of fat with the abundance *Staphylococcus* (number samples with detection of these genera).[44] In contrast, the total amount of protein was positively correlated with the abundance of *Anaerococcus, Bacillus* and *Peptoniphilus*.[44]

The concentrations of human milk oligosaccharides (HMOs) in BM is influenced by maternal blood type, the expression of different secretion proteins (fucosyltransferases) and maternal HIV infection status.[67,96] In one small study (11 participants), the concentration of HMOs was positively associated with the absolute abundance of *Bifidobacterium,* sialylated HMO with *B. breve,* non-fucosylated/non-sialylated HMO with *B. longum,* fucosylated HMO with *A. muciniphila* and fucosylated/silylated HMO with *S. aureus*.[57] In two other studies, (393, 50 participants respectively), the concentration of HMO in BM was reported not to influence the diversity of the BM microbiota.[33,67] The one study that reported maternal HIV infection status influences HMO concentration in BM hypothesised that this might contribute to the differences in the infant microbiota observed between HIV-exposed and HIV-unexposed infants.[67]

Association of the lipid profile with the microbiota revealed that concentrations of MUFAs were negatively associated with the relative abundance of Proteobacteria, while the relative abundance of *Lactobacillus* was positively correlated with concentrations of MUFAs (80 participants).[45]

Probiotics during pregnancy

Three studies (84, 125, 20 participants respectively) showed that probiotic administration during pregnancy did not affect the composition of the BM microbiota.[30,34,68] One study could isolate the probiotic bacteria administered to women in their BM.[34] The probiotics given to participants in the three studies were *L. rhamnosus* LPR and *B. longum* BL999 or *L. paracasei* ST11 and *B. longum* BL999; [31] *Lactobacillus rhamnosus* LPR and *Bifidobacterium longum* BL999 or *Lactobacillus paracasei* ST11 and *Bifidobacterium longum* BL999;[34] or a *Bifidobacterium* not further specified.[68]

Smoking

Smoking has been reported to affect the immunological components of BM.[97] One large study (393 participants) investigated the effect of smoking on the BM microbiota and did not observe any effect on the diversity or composition.[33]

Geographic location

Several studies have reported differences in the composition and core microbiota of BM in different geographic locations.[36,41,44,53] One study suggested that women in the USA might have less *Lactobacillus* and *Bifidobacterium* in their BM compared with women in Europe.[53] Another smaller study (50 participants) reported differences in the composition of BM between women in Italy and Burundi (Italy: predominance of *Abiotrophia* and *Alloiococcus* in colostrum and *Parabacteroides* in mature milk. Burundi: *Aquabacterium, Peptrostreptococcus* and *Serratia* in colostrum and *Dolosigranulum, Rhizobium* and *Weissella* in mature milk).[41] Another study (80 participants) that compared the microbiota composition of BM across four countries, found a higher relative abundance of *Enterobacteriaceae* and *Pseudomonadaceae* in South Africa, Firmicutes in Finland, *Streptococcus* in China and *Cutibacterium* and *Pseudomonas* in Spain.[45] *Lactobacillaceae* were found uniquely in samples from Finland, *Bifidobacteriaceae* only in samples from South Africa and *Enterococcaceae* in samples from all countries except China.[45] However, since DNA extraction was done in different laboratories, therefore these findings need to be interpreted with caution. Bacterial composition on family level has also been reported to differ amongst samples from different geographical regions in China and Taiwan (133, 89 participants respectively).[32,36]

Mastitis

Two studies (20, 50 participants respectively) observed that bacterial diversity was lower in BM of women with mastitis compared with healthy women.[27,43] During acute mastitis, *S. aureus* dominated the microbiota of BM, while during subacute mastitis it was *S. epidermidis*.[27] Furthermore, one study (50 participants) reported a lower abundance of anaerobic bacteria, *Acinetobacter, Clostridium, Eubacterium, Faecalibacterium* and *Ruminococcus* and a higher relative abundance of *Aeromonas, Enterococcus, Klebsiella, Pseudomonas, Ralstonia, Serratia* and *Staphylococcus* in women with mastitis.[43] In the one study that investigated this, archaeal DNA was absent in BM of women with mastitis, while it was found in all healthy women.[27]

Correlation with human cells

One study investigated the number of human cells in BM and did not find a correlation between the number of bacteria and the number of cells, suggesting that the bacteria in BM are not sensed as an infection by the immune system (21 participants).[44]

Collection and feeding method

One study (393 participants) showed that BM obtained by pump had a lower diversity, but a higher relative abundance of the potential pathogens *Enterobacteriaceae* and *Pseudomonas* and lower relative abundance of *Bifidobacterium*, while manually expressed milk had a higher relative abundance of *Gemellaceae, Nocardioides* and *Vogesella*.[33] Lower total bacterial loads were reported in aseptically collected samples.[47,50,51] One study also found a higher relative abundance of *Acinetobacter* in aseptically collected BM samples.[47]

Women who exclusively breastfed had a higher relative abundance of *S. parasanguis* compared with women who used mixed infant feeding (144 participants).[60]

Discussion

The studies in this review indicate that BM contains a largely diverse microbiota that is dominated by *Staphylococcus, Streptococcus, Lactobacillus, Pseudomonas, Bifidobacterium, Corynebacterium* and *Enterococcus*, but also contains archaea, fungi, eukaryotes and viruses.

There was some evidence that gestational age, delivery mode, biological sex, parity, IAP, lactation stage, diet, BMI, composition of BM, HIV infection, geographic location and collection/feeding method influence the composition of the BM microbiota. However, the majority of studies were small and the findings sometimes contradictory, meaning caution needs to be taken before drawing any firm conclusions on their relative influence.

Several studies reported that the BM microbiota is more diverse than the intestinal microbiota.[19,40,51,88] This raises the question, what is the significance of such a diverse BM microbiota? Bacteria are present in BM in sufficient numbers to colonise the infant intestine but they do not induce an inflammatory response in the mammary ducts.[44] Lactic acid bacteria (LAB) and *Parabacteroides* which are detected in BM, inhibit growth of pathogenic bacteria by production of antimicrobial compounds, such as bacteriocins (e.g. nisin), organic acids or hydrogen peroxide.[66,69,98–102] LAB have been shown to inhibit growth of *Bacillus cereus, E. coli, E. faecalis, E. sakazakii, Listeria monocytogenes, P. aeruginosa, S. aureus, Salmonella* serotype Enteritidis, *S. typhimurium*[103] and *Shigella sonnei*.[104] LAB also have been reported to improve the nutritional value of food and stimulate the immune system.[105] For some of the specific bacteria isolated from BM, explicit properties have been described. For example, *Parabacteroides* has been observed to reduce intestinal inflammation in animals models.[106] *Weissella* has been shown to inhibit biofilm formation[107] and to act anti-inflammatory.[108] *Alloiococcus* protects against colonisation with pathogenic bacteria.[109] *C. acnes* produces glycerol, which inhibits growth of *S. aureus.*[110] *S. epidermidis, S. salivarius, E. faecalis, Lactobacillus, Lactococcus* and *Leuconostoc* also suppress the growth of *S. aureus,* a major cause of mastitis.[66] Some women experience mastitis repeatedly, while others are not affected,[111] so it could be that differences in the composition of the BM microbiota contribute to this. One study showed that, even though *Haemophilus* was more abundant in BM of women who delivered vaginally, it was of higher importance in the BM of women who delivery by CS, where it acted as one of the main bacterial hubs in the microbiota network. Therefore, it is possible that, in addition to the abundance of microbes, the interactions between them, is important.[37] Crucially, animal models show that even transient exposure to bacteria can have health benefits, such as increasing IgA production.[112,113] Moreover, the BM microbiota likely also influences the composition of the BM (antibodies, immune cells and anti-microbial peptides etc.).

Currently, it is still unclear how microbes reach the BM. It has been suggested that mammary ducts become colonised by the

infant oral microbiota during suckling, as retrograde flow of BM into mammary ducts has been documented.[114] However, colostrum already contains bacteria before suckling has occured.[26] Furthermore, buccal administration of colostrum to low birth weight infants changed their oral microbiota compared with infants who were given standard care, suggesting that the BM might be responsible for colonising the infants mouth, rather than the opposite.[115] It has also been suggested that bacteria from the skin (such as *Corynebacterium, Cutibacterium* and *Staphylococcus*) colonise the mammary ducts. However, many anaerobic bacteria such as *Bacteroides, Bifidobacterium* and *Clostridium,* which are not found on skin, can be detected in BM.[25–27,29,39,43,57] Furthermore, one study showed that *Lactobacillus* present in BM are genotypically different from those detected on skin within individuals.[55] As many of the bacteria found in BM can also be found in the intestine, it is plausible that an entero-mammary pathway exists: intestinal organisms, or their DNA, can be transferred from the intestine to the mammary ducts. Translocation from the intestine manly occurs through gut-associated lymphoid tissues[116–119] and involves transfer of bacteria through dendritic cells and macrophages.[118–120] Translocation has been reported to increase in pregnant or lactating women.[88] For some bacteria (*Bacteroides, Bifidobacterium, Blautia, Clostridium, Collinsella, Cutibacterium, Enterococcus, Escherichia, Lactobacillus, Parabacteroides, Pediococcus, Staphylococcus, Streptococcus* and *Veillonella),* transfer from the maternal intestine into BM and the infant intestine has been shown.[29,51,88,121–123] Adding to the evidence for the existence of an entero-mammary pathway, is the fact that when *Lactobacillus* is administered as a probiotic to women, the same strain can be identified in BM,[124–126] as well as the finding that bacteria of commonly consumed foods can be isolated in BM.[29,41,127] Interestingly, it has been shown that even though mononuclear cells only contain low numbers of culturable bacteria, they harbour a much higher number of bacterial DNA. These same DNA signatures can be found in maternal faeces, BM and infant faeces.[88] DNA alone can be responsible for immune development. For example, unmethylated cytosine phosphate guanine (CpG) within bacterial DNA can stimulate Toll-like receptors.[128] Polyguanosine or guanosine cytosine-rich sequences that have been isolated from *Lactobacillus* can counter the effects of CpGs and can therefore act immune-suppressive.[129,130]

A further question that needs to be answered is the importance of the composition of the BM microbiota on infant health. The composition of the BM microbiota affects the composition of the intestinal microbiota in infants, which in turn plays a crucial for the development of the immune system.[38] *Staphylococcus* is one of the most abundant bacteria in BM. It has also been reported more abundant in faeces of infants who are breastfed compared with formula-fed.[131,132] Exclusively breastfed infants also have a higher relative abundance of *Bifidobacterium* in their faeces.[16] Furthermore, lower absolute abundance of *Bifidobacterium* in BM have been correlated with lower absolute abundance of *Bifidobacterium* in the infant intestinal microbiota,[17] which might allow stronger colonisation of *Bacteroides.*[133] High relative abundance of *Bacteroides* has been associated with the development of allergic diseases later in life.[20] A higher relative abundance of *Bacteroides* and *Clostridium,* and a lower relative abundance of *Bifidobacterium* and *Lactobacillus* have been associated with the development of allergic sensitisation, eczema or asthma.[20] A higher relative abundance of *Bacteroides* has also been associated with lower vaccine responses to oral rotavirus vaccine and a higher relative abundance of *Bifidobacterium* with higher vaccine responses to polio and tetanus.[24]

The limitations of this review are that the majority of studies were small and that the study designs were heterogenic (in particular that some of the studies collected BM aseptically and others did not). Results were often reported on different taxonomic levels (phyla, family, genus and species) making comparison across studies more difficult. Furthermore, many of the studies did not report antibiotic use in pregnancy, during delivery or during the lactation period. The microbiota analysis techniques also varied considerably. While culture only detects viable bacteria, molecular diagnostics also detect non-viable bacteria. It has been shown that bacteria in BM can either be in the free-living 'planktonic' stage or attached to human immune cells.[44] Attachment to human cells might decrease growth on culture.[44] Furthermore, culture-based methods reveal only a small part of fungal species and likely underestimate the proportions of anaerobic bacteria. However, with molecular diagnostics, results also varied depending on the use methods. For example, it has been suggested that 16s rRNA gene sequencing might underestimate the number of Gram negative bacteria.[134] Furthermore, universal primers are known to have a low amplification efficiency for bacterial genes containing high G+C content and might therefore underestimate *Bifidobacterium.*[135] *Bifidobacterium* are also hard to lyse and bead-beating during DNA extraction increases the yield. Furthermore, breast milk is a low microbial biomass sample, so it is crucial to take meticulous precautions to avoid contamination and identify microbial DNA signals from the environment or extraction and sequencing kits. For example, the findings that mothers who receive IAP have a higher bacterial richness and diversity in their breast milk microbiome compared with mothers who do not receive antibiotics, could be because antibiotics lead to lower total bacterial loads and therefore signals from contamination, e.g. bacteria found in DNA extraction or sequencing kits might be amplified more leading to a the detection of a higher diversity (contamination through the 'kitome').

In conclusion, BM contains a largely diverse microbiota, which is likely influenced by many internal and external factors. The BM microbiota likely has important implications for maternal and infant health. Several bacteria isolated from BM have been evaluated for use as probiotics.[103,104,136,137] Interventions such as to enhance the beneficial properties of BM or artificial milk offer exciting opportunities to positively impact infant health. Larger and better designed studies that include identification of fungi, archaea, eukaryotes and viruses, as well as the interaction between microbes are needed to maximise this opportunity.

Declaration of Competing Interest

The authors declare that they have no competing interests.

CRediT authorship contribution statement

Petra Zimmermann: Writing - original draft. **Nigel Curtis:** Writing - review & editing.

Acknowledgement

PZ is supported by a Fellowship from the European Society of Paediatric Infectious Diseases.

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