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REVIEW ARTICLE

Abstract

The intestinal microbiota plays a major role in infant health and development. However, the role of the breastmilk microbiota in infant gut colonisation remains unclear. A systematic review was performed to evaluate the composition of the breastmilk microbiota and evidence for transfer to/colonisation of the infant gut. Searches were performed using PUBMED, OVID, LILACS and PROQUEST from inception until 18th March 2020 with a PUBMED update to December 2021. 88 full texts were evaluated before final critique based on study power, sample contamination avoidance, storage, purification process, DNA extraction/analysis, and consideration of maternal health and other potential confounders. Risk of skin contamination was reduced mainly by breast cleaning and rejecting the first milk drops. Sample storage, DNA extraction and bioinformatics varied. Several studies stored samples under conditions that may selectively impact bacterial DNA preservation, others used preculture reducing reliability. Only 15 studies, with acceptable sample size, handling, extraction, and bacterial analysis, considered transfer of bacteria to the infant. Three reported bacterial transfer from infant to breastmilk. Despite consistent evidence for the breastmilk microbiota, and recent studies using improved methods to investigate factors affecting its composition, few studies adequately considered transfer to the infant gut providing very little evidence for effective impact on gut colonisation.

Keywords: microbiota, infant, breast milk, gut colonisation, systematic review

1. Introduction

There is increasing evidence for the role of the gut microbiota in many conditions and disorders, including allergy (Lynch and Boushey, 2016), obesity (Khan *et al.*, 2016), gut disease (Quince *et al.*, 2015) and neurological dysfunction (Wang and Kasper, 2014). Early microbial colonisation of the infant gut is likely to impact the composition of the gut microbiota in later life. Early development and microbial colonisation of the gastrointestinal (GI) tract also overlap

with critical periods for metabolic, immunological, brain and behavioural development (Hooper *et al.*, 2012; Houghteling and Walker, 2015; Marchesi *et al.*, 2016; Rinninella *et al.*, 2019; Vuong *et al.*, 2017).

While the human gut microbiota includes all microorganisms that live in the intestinal tract, including bacteria, archaea, viruses and fungi, the term gut microbiome refers to both the community of microorganisms and their theatre of activity including genetic material and metabolites (Berg *et al.*, 2020; Turnbaugh *et al.*, 2007; Ursell *et al.*, 2012). Together these contribute to protection against pathogens, education of the immune system, and many metabolic and physiological functions (Shreiner *et al.*, 2015).

Important established factors that impact the development of the gut microbiota in infancy are mode of delivery, gestational age at birth (Hill *et al.*, 2017; Moya-Perez *et al.*, 2017), and infant feeding (Backhed *et al.*, 2015; Fallani *et al.*, 2011; Murphy *et al.*, 2017; Penders *et al.*, 2005). Perinatal influences such as the use of antibiotics (Bokulich *et al.*, 2016; Nogacka *et al.*, 2017), maternal diet (Robertson *et al.*, 2017), hygiene practices and maternal prenatal stress (Foster *et al.*, 2017; Zijlmans *et al.*, 2015) are also important.

Among the many health benefits of exclusive breastfeeding for newborn infants, breastmilk promotes the selective proliferation of a characteristic gut microbiota, in addition to being a source of glycoconjugates, antimicrobials, bioactive proteins and other molecules (Chatterton *et al.*, 2013). It is now generally accepted that breast milk contains its own microbiota, reported to be dominated by *Staphylococcus, Streptococcus*, and *Cutibacterium* (formerly known as *Propionibacterium*) species and containing lactic acid bacteria and bifidobacteria (Fitzstevens *et al.*, 2017). As barriers to gut colonisation (gastric acid, bile acids and pancreatic enzymes) are likely to be less efficient in the first days after birth, the potential contribution of the colostrum microbiota is particularly relevant. Some similarities in the types of bacteria reported in breastmilk and infant faeces have been reported (Martin and Mayer, 2017; Martin *et al.*, 2012) but evidence of vertical transfer of bacteria via breast feeding to the infant gut resulting in early gut colonisation is very limited. Strong evidence would require following specific strains from breastmilk to infant gut. Murphy *et al.* (2017) reported only one mother infant pair with the same strains out of ten dyads studied. In addition, the source of bacteria detected in human milk and whether they survive in and contribute to colonisation of the infant's gut remains unclear.

Similarities in the microbiota of breast milk and the infant's gut may be explained by other mechanisms. Breast milk contains several nutritional and bioactive components, such as lactoferrin and oligosaccharides, which could influence the microbiota in breastmilk and the infant gut in a similar way. The infant may also influence bacteria populations in breast milk by transfer of oral microbiota during suckling (Ruiz *et al.*, 2019).

For proper evaluation of the impact of the breastmilk microbiota on infant gut colonisation several methodological issues should be considered. For analysis of human milk samples, the method of sampling, including avoidance of contamination, should be well described along with mode of birth and maternal factors (illness, medication and especially antibiotics) which may influence the microbial composition of human milk. Finally, the low abundance of bacteria in milk requires detailed information on microbiota analysis methods to allow consideration of contamination risk (Chong *et al.*, 2018). There is increasing evidence of potential contamination of samples during processing, including bacteria present in DNA extraction kits (recently termed 'kitome and splashome' (Olomu *et al.*, 2020). Ideally blank controls should be used at all relevant stages to account for this.

A systematic review of the published literature was therefore conducted to review the quality and agreement of studies designed to determine the composition of bacteria in colostrum and breast milk, and to review the scientific evidence for bacterial transfer from mother's milk to the infant and the possible impact on the early colonisation of the infant's gut.

2. Materials and methods

In this study we used five key stages to determine if bacteria can be transferred specifically from secreted breastmilk to the infant gut.

1. Identifying high quality information on the composition of the breastmilk microbiome collected with suitable breast cleaning techniques.
2. Evaluating the consistency of bacterial composition in breastmilk in different studies and the impact of key factors such as longitudinal changes (colostrum vs mature milk, early breastfeeding vs prolonged feeding); geographical differences; maternal body weight/body mass index (BMI); mode of delivery.
3. Considering those quality studies with circumstantial evidence of possible transfer that compare breast milk microbiota composition with that in the infant gut.
4. Identifying studies which looked at traceable markers of breastmilk bacteria – strains, antibiotic resistance, probiotics.
5. Considering possible back transfer from infant to mother's breast milk/tissues.

Systematic search

Searches were carried out using PUBMED, OVID, LILACS and PROQUEST from inception until 18th March 2020. Reference lists of relevant papers, including reviews, were searched for additional studies. An updated search was carried out (March 2020 to December 2021) in PUBMED only.

The search words included ((‘Microbiota’[Mesh] OR ‘Metagenome’[Mesh] OR ‘Dysbiosis’[Mesh]) AND ‘Anti-Bacterial Agents’[Mesh]) AND (‘Infant’[Mesh] OR ‘Infant, Extremely Premature’[Mesh] OR ‘Infant, Extremely Low Birth Weight’[Mesh] OR ‘Infant, Low Birth Weight’[Mesh] OR ‘Infant, Very Low Birth Weight’[Mesh] OR ‘Infant, Small for Gestational Age’[Mesh] OR ‘Infant, Premature’[Mesh] OR ‘Infant, Postmature’[Mesh] OR ‘Infant, Newborn’[Mesh] OR ‘Infant, Premature, Diseases’[Mesh]) AND (microbiota OR bacteria OR microflora OR microbes) AND (dysbiosis) AND (infant OR neonate OR baby) AND (health) AND (disease) AND (birth OR parturition) AND (breastmilk OR breast milk OR human milk) AND (breastfeeding OR breast feeding OR breastfed) AND (formula fed OR infant formula OR bottle fed OR bottle feed) AND (lactation). PROSPERO register CRD42017039875.

Titles and abstracts of studies identified by the search were screened by at least two independent investigators (EvdB, SO, MS, CvLB, CAE, JvD, CS) to identify those that evaluated the presence of bacteria in breastmilk. Study details from selected full-text reports were extracted into a spreadsheet for comparison in parallel by three independent investigators.

Papers were evaluated against the criteria below for inclusion and exclusion of manuscripts into the systematic review. We considered: (1) studies that characterised bacteria in breastmilk samples; and (2) those which attempted to follow the colonisation process from breast milk to the infant faeces by looking at specific organisms or species including probiotics. The PRISMA flow chart is shown in Figure 1.

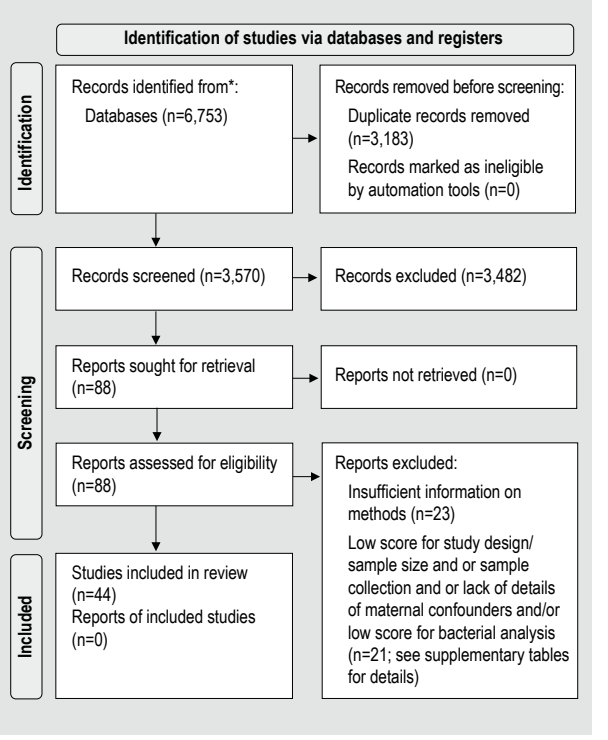

Figure 1. PRISMA 2009 flow diagram.

Inclusion criteria

1. Study and sample size: information on the number of samples and study participants; longitudinal sampling (ideally more than one breast milk sample collected from the mother), feeding setting (e.g. exclusive breast feeding) and details on timing (whether colostrum, transitional or mature milk samples were collected).
2. Sample collection and handling: detailed method of sample collection (e.g. by pump or hand; discard of first drops), precautions taken for contamination avoidance (hygiene practice before and during expression of milk sample) and any prevention of skin contamination (whether the skin was cleaned and with what before sampling); technical considerations, such as sample handling after collection including information on sample transport, storage and freezing.
3. Maternal confounders: essential information included mode of delivery, birth conditions, mastitis, desirable information included maternal health profile status, such as BMI, use of antibiotics and medications; and a statement on whether confounders were taken into account in statistical analysis or if data were excluded or analysed separately.
4. Sample handling, methodology and statistical analysis: information on sample handling ideally with a clear description of: timing between collection, storage and DNA extraction, storage conditions, DNA extraction kit, information on microbiota analysis methodology, statistical methods and where appropriate, bioinformatics. Finally, for the subset of papers that allowed assessment of bacterial transfer to the infant:
5. Bacterial transfer: confirmation of maternal origin of bacteria (were additional samples taken?); stage of lactation and timing (pre colostrum, colostrum or mature milk; details and timing of samples from infant (oral, faecal, meconium).

Based on the information collected, a quasi-risk of bias analysis was conducted as traditional risk of bias analysis was not feasible for a systematic review of this kind. This was mainly related to the lack of detail and consistency in the collection and sample handling as well as the

heterogeneity in reported outcomes. The paper quality was scored as high, medium, or low for items 1 to 4, as summarised above, by three independent reviewers and discrepancies between decisions were discussed with the whole group for a final agreement. All low graded papers (low score for items 1 to 3) were excluded from further detailed data extraction and evaluation. Data were extracted and assessed from 88 papers in total, and these were then discussed for quality of the evidence resulting in a final list of 44 papers (43 studies) for inclusion in the systematic review. Papers were scored for bacterial analysis by two reviewers (CAE, AG) considering sample processing and storage (samples should be stored at -80 °C quickly after collection), DNA extraction and/or bacteria identification methods, and analysis of DNA (pre-culture scored low). Studies that achieved a low score for these parameters were excluded from further analysis but were still considered for bacterial transfer if they used culture techniques for specific markers (strains of individual species, probiotic strains, antibiotic resistance) for transfer from mother's milk to the infant, in which case they were given a score of S (specific; 3 additional papers).

3. Results

Identification of high-quality data

88 papers were considered for detailed data extraction. Of these, 23 had insufficient required information. A further 21 papers scored low for one or more essential criteria and were excluded (Supplementary Tables S1-S6). The remaining papers addressed the identification of the microbiota in breast milk (Table 1 and Supplementary Tables S1-S6) and a subset of these also analysed infant faecal and/or oral samples to investigate possible microbiota transfer from mother to infant (n=15; Table 2 scoring high, medium or specific; for bacterial analysis see Supplementary Tables S2 and S3). Four studies specifically investigated the effect of a probiotic intervention on the transfer of microbiota into the breast milk of the mother collecting samples before and after treatment, but only one was of sufficient quality to be included for data extraction (Simpson *et al.*, 2018).

General considerations for all papers collecting breastmilk and colostrum

Study and sample size

The majority of studies recruited between 17 and 99 subjects (but this varied from 10 to >100) (Supplementary Tables S1-S6). The type of samples (e.g. colostrum, mature milk, breast skin swabs, infant faeces, infant oral swabs, maternal faeces), timing (before birth, colostrum, early milk only, repeated samples, up to 6 months) and numbers of samples collected from infants and mothers varied considerably (Supplementary Tables S1-S6). Most infants were healthy term-born infants, but no details were provided in several studies and some included preterm infants.

Milk sample collection details

In general, the methodologies for sample collection, processing and storage were poorly documented. Methods used to prevent skin contamination of the collected breastmilk included washing the breast with water, soap and water, chlorhexidine or iodine but some papers provided no information. Most studies rejected the first few drops of milk. Three studies (Kordy *et al.*, 2020; Pannaraj *et al.*, 2017; Sakwinska *et al.*, 2016) took extra samples to check for possible confounding by skin bacteria.

Maternal health confounders

Relative numbers of vaginal and caesarean births of the mothers varied considerably between studies and mode of delivery was not reported in five papers. Few papers mentioned maternal health and antibiotic use, nor whether these were taken into account in statistical analysis.

Sample handling and microbiota analysis

A range of different kits and methods were used to extract bacterial DNA (Supplementary Tables S2 and S4). The methodology used to analyse the microbiota varied from culturing, PCR to full genome sequencing.

Consistency of milk microbiota and factors affecting composition

Microbiota of pre-colostrum/colostrum

Studying the microbiota in mothers milk collected just before or in the first days after birth may provide the strongest evidence for possible transfer of microbiota to the infant. Only one study (Ruiz *et al.*, 2019) collected precolostrum. They reported the main phyla as *Firmicutes* (77%), *Actinobacteria* (11%) and *Proteobacteria* (6%) with *Streptococcaceae* [*Streptococcus*] and *Staphylococcaceae* [*Staphylococcus*] dominating at family and genus level. *Micrococcaceae* [*Rothia* and *Kocuria*], *Corynebacteriaceae* [*Corynebacterium*] and *Veilonellaceae* [*Veillonella*] usually associated with the mouth or skin were also detected along with *Bifidobacteriaceae* [*Bifidobacterium*].

The microbiota of colostrum was considered in ten studies. Mastromarino *et al.* (2014) looked specifically for lactobacilli and bifidobacteria and found these in all colostrum samples of mothers with term infants. Other studies looking more widely at the microbiota in colostrum reported less or no dominance of bifidobacteria with only Obermeyer *et al.* (2014) confirming significant populations. Sakwinska *et al.* (2016) detected low proportions of lactobacilli and

Table 1. High and medium scoring papers considered for composition of breast milk microbiota.[1]

Study	Study & sample size	Sample collection details	Maternal confounders	Bacterial transfer	Colostrum samples	Bacterial analysis
Boix-Amoros *et al.,* 2016	H	H	L[2]	–	yes	M
Browne *et al.,* 2019	H	H	H	–	–	M
Cabrera-Rubio *et al.,* 2012	H	H	M	–	yes	M
Cabrera-Rubio *et al.*, 2016	M	H	H	yes	–	M
Cortes-Macias *et al.,* 2021a	H	H	H	–	–	H
Cortes-Macias *et al.,* 2021b	H	H	H	–	–	H
Dave *et al.*, 2016	M	M	M	–	yes	M
Ding *et al.,* 2019	H	H	H	–	–	H
Dutta *et al.,* 2021	M	H	M	–	–	H
Fehr *et al.,* 2020	H	M	M	yes	–	M
Gonzalez *et al.,* 2021	M	H	M	–	–	M
Hunt *et al.,* 2011	H	H	L[3]	–	–	M
Jost *et al.,* 2013	M	H	H	–	yes	M
Jost *et al.,* 2014	M	H	H	yes	yes	H
Kordy *et al.,* 2020	H	M	H	yes	–	M
Kumar *et al.,* 2016	H	H	H	–	–	M
LeMay-Nedjelski *et al.,* 2020	H	M	M	–	–	H
Lugli *et al.,* 2020	H	M	L	–	–	H
Mastromarino *et al.,* 2014	H	H	M	yes	yes	M
Obermajer *et al.,* 2015	H	H	L[4]	–	yes	M
Ojo-Okunola *et al.,* 2019	H	M	H	–	–	H
Pace *et al.,* 2020	H	H	M	yes	–	M
Padihla *et al.,* 2020	H	H	M	–	–	H
Pannaraj *et al.,* 2017	H	M	M	yes	yes	M
Reyes *et al.,* 2021	H	M	M	–	–	M
Ruiz *et al.,* 2019	H	H	H	oral	yes	H
Sakwinska *et al.,* 2016	H	M	H	–	yes	M
Sanjulian *et al.,* 2021	H	M	M	–	–	H
Simpson *et al.,* 2018	H	M	M	–	–	H
Sinkiewicz *et al.*, 2008	M	M	L[5]	–	–	M
Treven *et al.,* 2019	H	M	H	–	–	H
Tuzun *et al.,* 2013	H	H	M	yes	–	M

[1] Data for studies not reaching quality criteria are in Supplementary Tables S2, S3 and S4). H = high quality scoring, M = medium quality scoring, L = low quality scoring.
[2] No mention of antibiotic use. Mode of delivery not taken into analysis.
[3] No mention of antibiotic use, mode of delivery and birth conditions.
[4] No mention of antibiotic use and mode of delivery.
[5] No mention of mode of delivery and birth conditions.

bifidobacteria in the colostrum of Slovenian women using aseptic (0.9%, 0.5%) and standard protocols (0.03%, 0.15%), respectively.

A high abundance of *Staphylococcus* and *Streptococcus* was detected in most studies. Obermajer *et al.* (2014) reported *Staphylococcus* (*S. epidermidis* and *S. aureus* and *Gemella* sp.) to be the most abundant with lower abundance of *Streptococcus oralis*, *Streptococcus pneumonia* and *Streptococcus salivarius.* There was also a high prevalence of *Enterobacteriaceae, Clostridium, Bacteroides-Prevotella* with *Enterococcus* found in 8.9% of samples. Cabrera-Rubio *et al.* (2012) reported dominance of *Weissella*, *Leuconostoc*, *Staphylococcus*, *Streptococcus*, and *Lactococcus.* Jost and colleagues (Jost *et al.*, 2013, 2014) reported staphylococci and streptococci as the predominant members of the *Firmicutes* phylum in breast milk, and a high relative abundance of *Pseudomonas* and *Ralstonia* from the *Proteobacteria.* There was a high relative abundance of *Bacteroidetes* mainly due to members of the *Flavobacterium* genus (4.4% mean relative abundance). Dave *et al.* (2016) reported a mean relative abundance of

Table 2. Studies considered for evidence of bacterial transfer from mother to infant via breastmilk.[a,b]

	Study and sample size	Sample collection details	Maternal confounders	Colostrum	Bacterial methods
Whole microbiota comparison					
Cabrera-Rubio *et al.,* 2016	M	H	H	-	M
Fehr *et al.,* 2020	H	M	M	–	M
Jost *et al.,* 2013[c]	M	H	H	yes	M
Jost *et al.,* 2014[c]	M	H	H	yes	H
Kordy *et al.,* 2020[d]	M	H	H	yes	H
Mastromarino *et al.,* 2014	H	H	M	yes	M
Pace *et al.*, 2021	H	H	M	–	H
Pannaraj *et al*., 2017	H	M	H	yes	H
Ruiz *et al.*, 2019	H	H	H	yes	H
Tuzun *et al*., 2013	H	H	M	yes	M
Individual species or strains					
Benito *et al.,* 2015	H	H	M	–	S
Makino *et al.,* 2011	M	H	H	–	S
Makino *et al*., 2015	H	H	L	–	S
Yan *et al.,* 2021	M	H	M	–	H
Zhang *et al.,* 2020	M	H	M	–	H

[a] For details go to Supplementary Tables.
[b] H = high quality score; M = medium quality score; L= low quality score. S = means used single strains or species relevant to establishing transfer even if used culture techniques.
[c] Data of same 7 mothers and split in 2 papers.
[d] Probiotic intervention studies.

73.8% for *Streptococcus* followed by *Staphylococcus* at 10.9%. In the study of Boix-Amoros *et al.* (2016), *Staphylococcus* was the most common genus, followed by *Acinetobacter,* whereas Sakwinska *et al.* (2016) reported a high abundance of *Acinetobacter* only in samples collected without a stringent aseptic approach. Pannaraj *et al.* (2017) found alveolar skin was the main source of *Staphylococcus* and *Streptococcus* in milk while the dominant phylum was *Proteobacteria* (*Moraxellaceae*, *Enterobacteriaceae*, and *Pseudomonadaceae*) (Jost *et al.*, 2014; Mastromarino *et al.*, 2014; Pannaraj *et al.*, 2017; Ruiz *et al.*, 2019).

Longitudinal changes in microbiota composition (mature milk)

As breastfeeding continues across the first year of life the breastmilk microbiota composition may change. Several studies included in this review collected samples at different timepoints, however they varied considerably in the number of mother infant dyads followed, the timing of each sample and used different bacterial and bioinformatic analysis. This makes it difficult to combine and compare data between studies.

Mastromarino *et al.* (2014) focussed on lactobacilli and bifidobacteria. They found no differences in bacterial numbers between colostrum and mature milk, but their levels were positively correlated to faecal lactoferrin. Sakwinska *et al.* (2016) found very low proportions of lactobacilli and bifidobacteria in samples collected with strict aseptic technique. The highest abundance was for staphylococci and streptococci. The most abundant genera in the longitudinal milk samples collected by Hunt *et al.* (2011) were *Streptococcus, Staphylococcus, Serratia* and *Corynebacterium*, with much lower abundance of eight other genera. Stability and dominance of bacterial communities differed between women but were more stable within individuals.

Simpson *et al.* (2018) analysed 472 breastmilk samples from 252 women in Norway collected at 10-d and 3-months postpartum. At both timepoints, the microbiota was dominated by Streptococcus and Staphylococcus genera but there was lower relative abundance or Staphylococcus genus at 3 months with more species, diversity and more operational taxonomic units (OTUs) from the genera *Rothia, Veillonella,Granulicatella* and *Methylbacterium.*

Browne *et al.* (2019) collected milk samples at 2, 6 and 12 weeks postpartum. *Firmicutes* remained the most dominant phylum between 2 and 12 weeks postpartum, but abundance decreased over time (87 to 58%). At 2 weeks

postpartum, *Proteobacteria* was the second most abundant phylum and increased over time (8 to 22%). The relative abundances of *Bacteroidetes* and *Actinobacteria* were very low (<2.5%), although the *Bacteroidetes* increased slightly at later timepoints. Shannon diversity increased over time, with an increase of *Lactobacillus* and other minor genera and a decrease in *Staphylococcus*.

Cabrera-Rubio *et al.* (2012) reported that *Weissella*, and *Leuconostoc* were the most common genera in mature milk followed by *Staphylococcus*, *Streptococcus* and *Lactococcus. Veillonella*, *Leptotrichia* and *Prevotella*, usually seen in the oral cavity, increased significantly in samples taken around 1 and 6 months after birth. However, this was not confirmed by Mastromarino *et al.* (2014) who reported no change in these bacteria over the course of lactation. Pannaraj *et al.* (2017) studied breastmilk over 12 months and found little change in bacterial relative abundance in the first six months. *Proteobacteria* (*Moxaxellaceae*, *Enterobacteriaceae* and *Pseudomonadaceae*) were the dominant phylum. There was no difference in α-diversity (within individuals) over the course of lactation, but β-diversity (between individuals) increased in the first 6 months after delivery and decreased thereafter.

Boix-Amoros *et al.* (2016) collected longitudinal samples at day 5 (colostrum), 6-15 (transitional) and >15 days up to 1 month (mature) in 21 women and three times with a 1-2 weeks interval (not further specified) in 16 women. Although some differences in breast milk bacteria load were observed at different timepoints there was no significant changes over the course of lactation. For colostrum, transitional and mature milk the most common genera were *Staphylococcus*, followed by *Acinetobacter* in colostrum, *Pseudomonas* and *Streptococcus* in transitional and *Acinetobacter* in mature milk. Bacterial diversity did not change significantly over time. *Finegoldia*, *Streptococcus, Corynebacterium*, *Staphylococcus*, *Acinetobacter*, *Peptoniphilus* and *Pseudomonas* genera were detected at all timepoints sampled. *S. epidermidis* was the most common staphylococcus. *S. aureus* was not detected. Bifidobacteria were detected at low levels, but the authors report that the detection levels could have been influenced by the low amplification efficiency of their adapted universal 8F and 785R primers when there is a high G+C content. Diversity and richness of the bacterial populations were not affected by bacteria load.

Sanjulian *et al.* (2021) considered mothers and infants breastfeeding for up to 5 years. As the duration of breastfeeding increased the dominant species changed; *Actinobacteria* and *Bacteroidetes* increased but the level of *Firmicutes* was largely unchanged over lactation. The α-diversity increased with greater variation between individuals (β-diversity).

Geographic differences in the composition of breastmilk microbiota

Several studies compared breastmilk microbiota in samples collected from mothers in different countries. Kumar *et al.* (2016) compared breastmilk samples at 1 month from mothers in China, South Africa, Finland and Spain (n=20/country). Using redundancy analysis (RDA) with OTUs they found milk from mothers in Spain and South Africa had more diverse interindividual microbial profiles than mothers from Finland and China. Milk microbiota composition differed significantly between the countries. For example, on a phylum level in mothers who delivered vaginally, Finland had higher counts of *Firmicutes* and lower levels of *Proteobacteria* compared to the other countries. *Proteobacteria* were more prevalent in South African women, while Spanish women had higher *Bacteroidetes* levels compared to the other countries. Chinese women had the highest levels of *Actinobacteria*. At the genus level, *Streptococcus* was higher in Chinese mothers' milk. The milk of Spanish mothers had higher levels of *Propionibacterium*, and *Pseudomonas*. Analysis at OTU level indicated more diverse microbial communities, regardless of the mode of delivery in Spanish and South African compared to Finnish and Chinese women. While 23 phylotypes constituted the core milk microbiota across all the countries, *Lactobacillaceae* were uniquely found in Finish samples, *Bifidobacteriaceae* in South African, while *Enterococcaceae* were found in mothers' milk from all countries except China. By comparing milk from 89 mothers from 11 regions in China, Ding *et al.* (2019) showed the microbiota profile is also highly region-specific. Day 42 postpartum milk from north and northwest China had higher α-diversity than other regions. The genera *Staphylococcus*, *Streptococcus*, and *Enterococcus* were dominant in all samples, although in different relative abundance across regions. There was a high occurrence of *Lactobacillus* (mainly *Limosilactobacillus reuteri* (formally *L. reuteri*) and *Lactobacillus gasseri*) in samples from the north and northwest. Finally, Sinkiewicz and Ljunggren (2009), collected samples from women in seven different countries (Sweden, Denmark, Israel, South Africa, Japan, South Korea and Peru) in rural or urban areas with focus on lactobacilli. *Limosilactobacillus reuteri* was found in samples from women around the world, with the highest colonisation frequency in Japanese women. Total lactobacilli were higher in samples from South Korea and Japan. There was no difference between rural and urban areas although the number of samples collected from rural area was lower than intended, and median lactobacilli counts was higher.

Impact of body weight and body mass index

Cabrera-Rubio *et al.* (2012) reported that the colostrum microbiota of mothers with obesity tended to be less diverse than that of normal weight mothers. However, Dave *et al.*

(2016) found a higher diversity of colostrum microbiota with increasing pre-pregnancy maternal BMI and a lower relative abundance of *Streptococcus.*

In another study (Kumar *et al.*, 2016), *Firmicutes* in breastmilk at one month were positively associated with pregestational BMI, in more than 98% of the women, irrespective of their country. In Chinese mothers, prepartum BMI did not associate with any dominant genera, while postpartum BMI was negatively correlated with *Lactobacillus* and positively with *Staphylococcus* (Ding *et al.,* 2019).

Cabrera-Rubio *et al.* (2012) compared the milk of normal-weight mothers with that of mothers with obesity and observed that bacterial composition was more homogenous in milk samples from women with obesity. and was affected by excessive weight gain during pregnancy. Higher maternal BMI was related to elevated total bacterial counts and higher numbers of *Lactobacillus* in colostrum, but with lower numbers of *Staphylococcus* and *Bifidobacterium* in milk samples collected at 6 months postpartum (Cabrera-Rubio *et al.*, 2012). Excessive weight gain was associated with higher *Staphylococcus,* in particular *S. aureus,* in samples taken 1 month after birth as well as higher amounts of *Lactobacillus* and lower amounts of *Bifidobacterium* in samples taken 6 months postpartum (Dave *et al.*, 2016). Similar results were found by Cortes-Macias *et al.* (2021b), in a study of the breastmilk microbiota of 136 women at one month. This was influenced by pregnancy weight gain and pre-pregnancy BMI, as well as exclusive breastfeeding vs mixed feeding. Mothers who were normal weight before pregnancy and breast fed exclusively had greater breastmilk microbiota α-diversity and higher abundance of *Bifidobacterium* genus.

Mode of delivery

Only a few studies specifically evaluated the impact of mode of delivery on breastmilk microbiota composition. Sakwinska *et al.* (2016) and Ojo-Okunola *et al.* (2019) did not observe any impact of delivery mode on the microbiota of breastmilk. Cabrera-Rubio *et al.* (2012) found milk from mothers with an elective C-section, but not from non-elective mothers, had a different bacterial community than milk from mothers with vaginal delivery suggesting that stress or hormonal signals may influence microbial transmission to milk. Kumar *et al.* (2016) reported that mode of delivery was associated with the milk microbiota profile 1 month postpartum, but the impact differed by country which could be related to the different (country specified) guidelines for antibiotic use for C-section delivery. On a phylum level, higher levels of *Proteobacteria* were observed in the milk of the Spanish and South African women who had a C-section that included antibiotic treatment. In contrast, samples from vaginal delivery and C-section were relatively similar in Finnish mothers, who did not receive antibiotic treatment.

Cabrera-Rubio *et al.* (2016) found higher diversity in single milk samples at 1 month from mothers with vaginal delivery than those with C-sections. The Chao richness index indicated an estimated 500 species-level OTUs in breast milk from six mothers with vaginal delivery, but only 250 OTUs in four mothers after C-section. Higher relative abundances of *Staphylococcus* and lower abundances of *Streptococcus* were found in milk from mothers with C-section compared with those with vaginal delivery. In another study, Ding *et al.* (2019) found a higher abundance of *Enterococcus* and a lower abundance of *Lactobacillus* in milk from mothers with C-section.

Evidence for bacterial transfer between mother's milk and the infant mouth/gut

Confirmation of bacterial transfer to the infant and potential colonisation was possible only if sufficient details of infant stool (or oral sample) analysis were available, including a check on the robustness of the data, for instance by collecting samples other than milk. Fifteen studies qualified for assessment (Table 2 and Supplementary Table S3). Only six studies compared strains of bacteria or specific markers such as antibiotic resistance profiles found in human milk and infant faeces directly (Benito *et al.*, 2015; Jost *et al.,* 2013; Kordy *et al.*, 2020; Makino *et al.*, 2015, Simpson *et al.,* 2018; Treven *et al.,* 2019). Of these, the studies by Simpson *et al.* (2018) and Treven *et al.* (2019) looked at the apparent transmission of probiotic strains provided by maternal (oral) intervention from mother to infant. A further study (Togo *et al.*, 2019) looked at the methanogenic archaea rather than bacteria so will not be considered here, but only in the discussion. The remaining studies simply compared the overall microbiota profile between mother and infant, identifying similarities in microbiota profiles present in human milk and infant faeces and/or mouth (Jost *et al.*, 2013; Kordy *et al.*, 2020; Mastromarino *et al.*, 2014; Pannaraj *et al.*, 2017; Ruiz *et al.*, 2019; Tuzun *et al.*, 2013) which is not convincing evidence of transfer. Two studies also considered transfer between mother and infant in both directions during sucking via the oral microbiota (Ruiz *et al.*, 2019; Treven *et al.*, 2019).

Four studies collected infant faeces at similar time points to the collection of milk samples (Benito *et al.*, 2015; Mastromarino *et al.*, 2014; Pannaraj *et al.*, 2017; Tuzun *et al.*, 2013). Benito *et al.* (2015) focussed on the presence of *S. aureus* in 12 out of 21 faecal samples, 2 on day 7, 3 on day 14 and 10 around day 35 after birth. *S. aureus* was detected in both milk and infant faeces of 6 mother infant pairs, but potential transfer of specific strains from mother to infant via breastmilk was identified for only four cases. Mastromarino *et al.* (2014) reported a significant positive

relationship between breastmilk and faecal bifidobacteria levels in full term infants at birth. Both lactobacilli and bifidobacterial levels increased between birth and 1 month in the infant faeces. Bifidobacteria levels were not significantly different at 1 month, but lactobacilli levels were significantly lower in preterm born compared to term infants. Pannaraj *et al.* (2017) reported distinct and different bacterial communities in milk, alveolar skin and infant faecal samples by Principal Coordinates Analysis (PCA). Bacterial diversity in infant stool increased by age and was influenced by the percentage of daily breastfeeding events in a dose dependent manner. Using a source tracking approach (Langille *et al.*, 2013), in which bacterial DNA sequences from different samples from the mother and infants were compared, the contribution of breastmilk bacteria to faecal bacterial composition was calculated to be 27.7% (standard deviation (SD) 15.2%) and that of the alveolar skin to 10.4% (SD 6.0%) in exclusive breast-fed infants during the first 30 days of life and contributions decreased after. Bacterial communities appeared to be mother-infant pair specific. 26 of 478 OTUs were more significantly shared between mother infant pairs than between random pairs. Tuzun *et al.* (2013) reported higher levels of *Bifidobacterium adolescentis, Bifidobacterium longum* and *Bifidobacterium bifidum* in faecal samples taken between 14 and 28 days after birth from children without jaundice compared to breast fed children that did develop jaundice. They reported a significant negative association between serum bilirubin levels and these three bacterial species, but a significant correlation between milk and faeces bacteria was shown only for *B. bifidum*. Also, in the study of Makino *et al.* (2015), *B. bifidum, Bifidobacterium breve* and *B. longum* subspecies *longum* were the only species that could be isolated from both human milk and infant faecal samples, respectively in 3, 11 and 5 mother infant pairs. They identified transfer of these strains by isolation on TOS propionate agar and genotyping by means of multilocus sequence typing (MLST) which identified 7 loci that showed the same sequence. However, it is not clear if these strains appeared in milk before infant faeces. Bifidobacteria were identified in 21 faecal samples but only 8 milk samples. Again, *B. breve, B. adolescentis* as well as *B. longum* were present in milk and faeces, and in addition, also *Bifidobacterium pseudocatenulatum* was found in faeces only and an overlap between milk and faeces samples was found for 8 out of 23 mother infant pairs only.

Jost *et al.* (2014) identified *Bifidobacterium breve* as the most appropriate marker for demonstrating vertical mother-neonate transfer via breast milk. Further, sequencing of the 16S rRNA genes showed that corresponding isolates (previously grown on selective culture media) between mothers' milk and their infant faecal samples were *S. epidermidis*, *B. longum*, and *Lacticaseibacillus casei* (formerly known as *Lactobacillus casei*) strains supporting colonisation from human milk. Zhang *et al.* (2020) considered lactobacilli phylotypes and Yan *et al.* (2021) from the same group studied bifidobacteria phylotypes in infants' faeces and breastmilk. There was evidence of similar lactobacilli phylotypes in mothers' milk and infant faeces but less clear evidence for bifidobacteria.

Two high/medium scoring studies collected colostrum samples and infant faeces over the first few days after birth (Mastromarino *et al.*, 2014; Pannaraj *et al.*, 2017). Again, most studies focussed on the possible transfer of bifidobacteria using a species comparison approach rather than identification of specific bacterial strains using state-of-the-art technology.

Three studies suggested transfer of bacteria between mother's milk and their infant, especially for oral bacteria. Ruiz *et al.* (2019) compared the microbiota of pre-colostrum with that detected from swabs of the infant mouth (15 mother-infant dyads. 16S rRNA sequencing, bacterial isolation, and whole genome sequencing (WGS) were used followed by comparative genomics of those isolates that apparently were shared between the same mother-infant pair. The bacterial profile of precolostrum, secreted by women at the end of pregnancy, was very similar to that in mature milk, (dominated by *Staphylococcus* species and other species associated with the oral cavity, such as *Streptococcus, Fusobacterium, Veillonella* or *Porphyromonas*). Makino *et al.* (2011) focused on *Bifidobacterium longum* subsp*. longum* and 11 strains of this species were monophyletic for the faeces of maternal and infant dyads. However, another study by Makino *et al.* (2015) identified *Bifidobacterium* strains that appeared first in infant faeces before being detected in human milk suggesting reverse transfer. This was supported by Treven *et al.* (2019) who found that probiotics given to the infant appeared in breast milk, so two-way transfer must also be considered.

4. Discussion

This systematic review aimed to understand the composition of the breast milk microbiota and the factors influencing this. More importantly this study aimed to explore the evidence for transfer of bacteria from mother to infant through breastfeeding which could result in infant gut colonisation.

Proof of bacterial transfer from mother to infant is very difficult to establish conclusively. To establish transfer it is necessary to have clear differentiation of specific strains in breastmilk that can be traced into the faeces of the infant (Benito *et al.*, 2015; Kordy *et al.*, 2020) or markers such as antibiotic resistance or resistome (Parnanen *et al.*, 2018). Very few studies have considered this level of interrogation and it is not possible to achieve this with many of the techniques used in studies published so far. Evaluation

of the long-term impact of any bacterial transfer on the developing gut microbiota of the infant would require adequate and repeated sampling and analysis of the bacteria in breastmilk and infant faeces over a long period of time. Nutritional/supplemental intake of pro- and prebiotics by a mother could be hypothesised to influence the microbiota in her breast milk (Maldonado-Lobon *et al.*, 2015; Padilha *et al.*, 2020). Consequently, probiotic intervention studies in the mother may help to understand what determines the bacterial levels and species in human milk, and whether this impacts the infant gut microbiota (Dotterud *et al.*, 2015; Mastromarino *et al.*, 2015). This is an important area for future research development.

There have been many studies published on the breastmilk microbiota over the past decade and this has coincided with a revolution in the technologies to analyse and characterise complex microbiomes resulting in next generation sequencing and new bioinformatic approaches. In more recent years these techniques have also come under scrutiny. It has become clear that DNA extraction techniques vary in their yield and quality, but along with sampling processes, they can also introduce contamination which is particularly evident in low biomass samples. Moreover, the storage of samples below -80 °C or even at -80 °C for prolonged periods can lead to disproportionate degradation of DNA from individual phyla, groups and species (Carruthers *et al.*, 2019; Gorzelak *et al.*, 2015). In this systematic review we applaud much of the early research from several laboratories that pioneered and introduced the concept of the breastmilk microbiota and promoted consideration of this potentially very important colonisation route for the infant gut (Supplementary Tables S5 and S6). However, for the purpose of establishing whether transfer of microbiota via breastmilk results in infant colonisation, only those studies which met our criteria for high (H) or medium (M) quality for bacterial analysis, study size, and sampling details were included. Studies that used specific techniques such as strains of species cultured from samples or markers of specific strains or traits which can trace colonisation more closely were also included. Clearly, there may be mechanisms/colonisation routes other than breastmilk transfer whereby similar bacterial profiles in breastmilk and infant faeces can be explained.

Although methodological details were provided in the papers selected in this review, many studies did not address possible contamination during collection or sample processing. Some studies used mechanical or electric pumps to sample the breast milk and others manual expression from the breast. Differing pumping techniques may influence the bacterial composition of the milk (Reyes *et al.*, 2021) and transfer of bacteria to the infant gut (Fehr *et al.*, 2020). This needs to be studied further.

The latest research looking at the concept of a normal placental microbiome has highlighted the importance of negative controls at all stages from sample collection to DNA analysis (De Goffau *et al.*, 2019) which must also be considered for the breast milk microbiome (Olomu *et al.*, 2020). The storage conditions of the samples and time to DNA extraction is also critical (Angebault *et al.*, 2018; Jenkins *et al.*, 2018; Wu *et al.*, 2019). Lyons *et al.* (2021) reported that if DNA extraction could not happen directly from fresh milk samples, then storage by freezing at -80 °C had the least impact on microbial DNA in human milk samples. The choice of DNA extraction kit can also affect final results due to differences in methodology and also contamination (Ojo-Okunola *et al.*, 2019; Salter *et al.*, 2014). When five different kits were used to analyse a mock breastmilk microbiome as well as human milk samples (Douglas *et al.*, 2020), differences in DNA yield, purity and sequencing depth were reported as well as contamination of samples by bacteria similar to those reported in previous studies as breastmilk microbiota. A similar comparison of four further DNA extraction kits (Cheema *et al.*, 2021) also reported variable levels of contamination and efficiency of bacterial DNA recovery from breast milk samples. Milk fat globules may interfere with the extraction of some species (Sun *et al.*, 2019) and this was not solved by centrifugation (Stinson *et al.*, 2021).

In this review, we included papers that used adequate cleansing of the breast before sample collection and aseptic techniques during processing, but very few ran negative controls for DNA extraction and analysis. In addition, we rejected several studies which stored samples at -20 °C for an extended period before DNA extraction or did not state storage time at -20 °C before DNA extraction (Lackey *et al.*, 2019).

We concentrated on studies that provided good evidence of a microbiota in breastmilk that comes from inside the breast ducts rather than the bacteria in breastmilk as ingested without breast cleansing which may be also seeded by bacteria on the skin of the breast. Breast skin bacteria could, of course, be part of the normal bacterial transfer process (Pannaraj *et al.*, 2017), but we were interested in bacteria specifically from breastmilk itself. Bacterial RNA has been reported in milk cells, breast ducts and maternal blood (Perez *et al.*, 2007) providing some evidence of potential secretion of bacteria into human milk. Several studies have demonstrated the presence of a substantial microbiota in human milk, despite a considerable variation in methodology concerning DNA extraction, sample analysis and statistical/bioinformatic approaches. The exact composition of the breastmilk microbiota varied between mothers and between studies which is very likely due in part to the different methods used and geography, but many studies reported a dominance of *Staphylococcus* and *Streptococcus*, *Lactococcus*, with *Pseudomonas* and

some lactobacilli and bifidobacteria (Lugli *et al.*, 2020) in colostrum and early milk. In addition, evidence of the methanogenic archaea (*Methanobrevibacter smithii* and *Methanobrevibacter oralis*) in human colostrum and milk has been reported (Togo *et al.*, 2019), so the whole microbiota and not just bacteria need to be considered.

Several longer-term studies considered in this review reported changes in bacterial counts over the course of lactation (Browne *et al.*, 2019; Pannaraj *et al.*, 2017), the longest for up to 5 years (Sanjulian *et al.*, 2021). There was some inconsistency between studies in the change in diversity of the microbiota over time. More recently (Lyons *et al.*, 2022) the milk microbiota of 80 lactating mothers was studied over 6 months, *Staphylococcus, Streptococcus, Pseudomonas, Acinetobacter, Bifidobacterium, Mesorhizobium, Brevundimonas, Flavobacterium,* and *Rhodococcus* genera predominated in milk samples over 24 weeks, but alpha diversity decreased over time with the biggest difference seen between 8 and 24 weeks. Maternal BMI (Ding *et al.*, 2019; Kumar *et al.*, 2016) or gestational weight gain (Cabrera-Rubio *et al.*, 2012; Dave *et al.*, 2016) and gestational age (term vs preterm) (Mastromarino *et al.*, 2014) were also considered and recent studies have confirmed the impact of these confounders (Cortes-Macias *et al.*, 2021b) but the evidence is still limited and the contribution of each of these factors needs to be further assessed.

The direction of transfer between mother and infant also needs to be assessed carefully. Treven *et al.* (2019) considered if the infant oral microbiota in saliva colonised the breast tissues and ducts rather than the other way around. Probiotic bacteria fed to infants were subsequently detected in their mother's milk. So reverse transfer from the infant's mouth to the breast skin and ducts must indeed also be considered.

If it is established that breastmilk bacteria can colonise the infant gut, the factors which determine the breastmilk microbiota, and the skin microbiota of the breast need to be considered. Many factors are likely to impact the composition of the breastmilk microbiota (Figure 2). Some of these (BMI and pregnancy weight gain, mode of delivery, hygiene) have been considered by the papers evaluated in this systematic review. And several recent studies have considered the potential impact of maternal diet (protein, polyunsaturated fatty acids (PUFA), pre- and probiotics), breastmilk composition (docosahexaenoic acid (DHA), human milk oligosaccharides-HMOs), and antibiotics for their impact on the breastmilk microbiota.

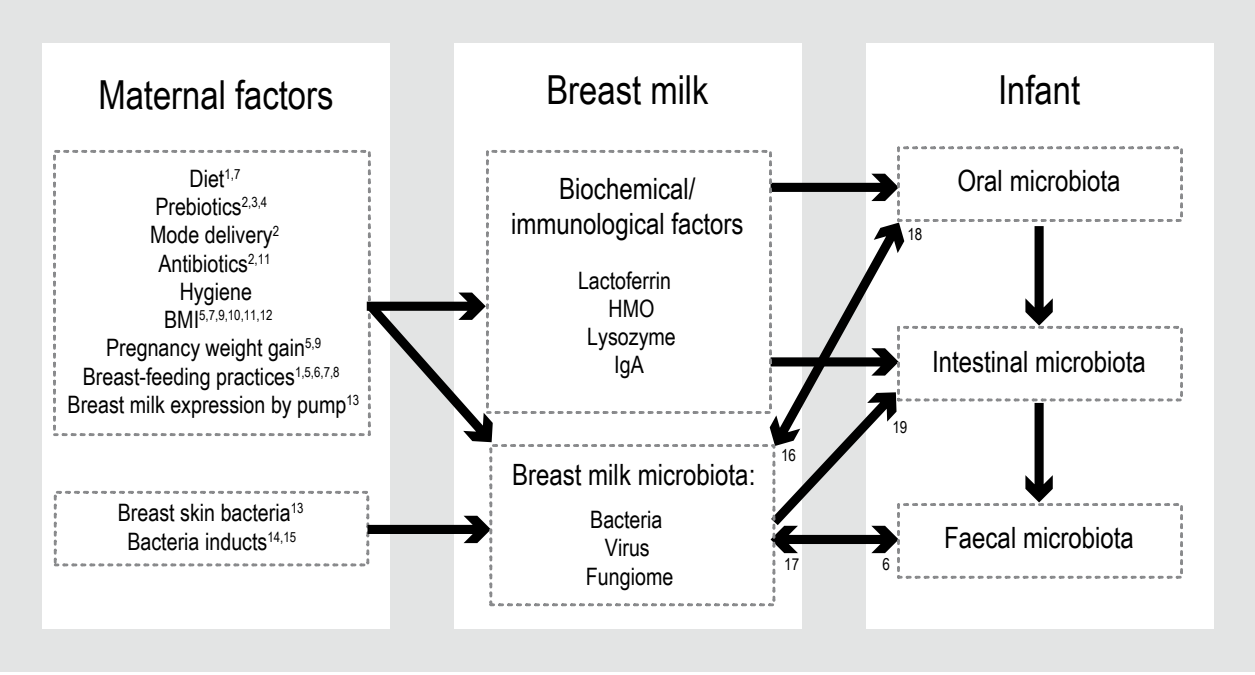

Figure 2. Main factors which could influence the breast milk microbiota and transfer between mother and infant. Evidence for impact of different factors and direction of transfer: [1] LeMAy-Nedjelski *et al.*, 2020; [2] Cortés-Macias *et al.*, 2020; [3] Padilha *et al.*, 2020; [4] Kongnum *et al.*, 2020; [5] Cortés-Macias *et al.*, 2021; [6] Fehr *et al.*, 2020; [7] Sanjulián *et al.*, 2021; [8] Gonzalez *et al.*, 2021; [9]Cabrera-Rubio *et al.*, 2012, [10] Dave *et al.*, 2016, [11]Kumar *et al.*, 2016, [12]Ding *et al.*, 2019, [13] Reyes *et al.*, 2021; [14] Pannaraj *et al.*, 2017; [15] Perez *et al.*, 2017; [16] Treven *et al.*, 2019; [17] Makino *et al.*, 2015; [18] Ruiz *et al.*, 2019; [19] few conclusive studies looking at strains, most studies looked at associations – see main text.

Cortes-Macias *et al.* (2021a) collected 120 milk samples from Spanish mothers, 7-15 days after birth. *Staphylococcus* populations were associated with higher carbohydrate intake and lower total protein. *Streptococcus* populations were associated with higher total protein intake, higher eicosapentaenoic acid (EPA), and docosapentaenoic acid (DPA), selenium and zinc. *Bifidobacterium* levels were associated with higher intakes of carbohydrate and polyphenols and with lower dietary lipids, mainly lower monounsaturated fatty acids (MUFA), and PUFA. In a study of 93 milk samples at 3 months postpartum from women with high rates of gestational glucose intolerance (LeMay-Nedjelski *et al.*, 2021), maternal intake of PUFA and grain fibre were associated with increased α-diversity. Fibre was associated with higher *Acinetobacter* and reduced *Streptococcus* and *Gemella*. MUFA intake was associated with *Acinetobacter* and *Gemella,* whereas PUFA intake was negatively associated with *Acinetobacter* (Sanjulian *et al.*, 2021). These authors also found significant correlations between maternal intake of vegetables and breastmilk *Staphylococcus* and *Firmicutes*, fish and seafood intake with *Bacteroidetes* (*Bacteroides* and *Prevotella*), whereas nut intake was negatively correlated with the ratio of *Firmicutes/ Bacteroidetes.* These potential dietary effects need to be confirmed and further explored.

Human milk DHA levels were related to milk *Proteobacteria* levels, conjugated linoleic acid correlated positively with *Staphylococcus,* whereas *Streptococcus* was negatively associated with trans palmitoleic acid in breastmilk (Sanjulian *et al.*, 2021). However, the bifidobacterial species found in human milk were not those that possessed the strongest ability to metabolise HMOs (Lugli *et al.*, 2020).

Breastmilk is likely to be a key source of ingested bacteria in the first few days of life and transfer via colostrum may be most important during this period. Over the first year of life, the bacteria in breastmilk may also influence gut function and development and several studies considered changes in the microbiota of breastmilk over time. However, whilst in the first days after birth there are reduced barriers to colonisation, such as lower secretion of gastric acid, pancreatic enzymes and bile acids, the microbiota in mature breastmilk at later periods of lactation (when these barriers are more efficient) may have less impact on gut colonisation. For successful transfer and survival in the infant gut, bacteria may need greater resistance properties similar to those of probiotic bacteria. There have been several studies which aimed to isolate probiotic contenders in breast milk. Riaz Rajoka *et al.* (2017) found two out of seven isolates of *L. rhamnosus* from breastmilk had good survival under simulated gut conditions indicating the possibility of survival in the gastrointestinal tract. More recently, Damaceno *et al.* (in press) identified four potential breastmilk derived probiotic strains, two *L. rhamnosus* and two *Leuconostoc mesenteroides* which were able to colonise the gut of germfree mice.

A major obstacle to establishing if the breastmilk microbiota influences infant gut colonisation is the lack of standardisation in methodologies and the information reported in different studies. Ideally this would include details of sample collection and contamination control, clear consideration of confounding factors, use of appropriate storage conditions (-80 °C; as soon as possible after sampling), early and consistent timing for DNA extraction (rarely reported in studies), use of appropriate negative process controls at all points of potential contamination and mock communities. Several studies published after the final date of our systematic review have instigated at least some of these changes. Moreover, strain level identification is essential to confirm transfer from mother to infant. Most of the more recent articles use DNA sequencing methodology based on the amplification of different hypervariable regions, e.g. V1-V3, V1-V5, V3-V4, etc., of the ribosomal gene 16SRNA, followed by Next Generation Sequencing (NGS) using Illumina procedures. However, the use of amplicons of about 500 bp for amplification of the V3 and V4 regions or other regions of the 16S ribosomal RNA gene, does not allow precision at the species level.

In conclusion, while there is considerable evidence that there is a diverse microbiota in human breastmilk across the period of breastfeeding with some consistency in composition, there are only limited data to support its role in colonisation of the infant gut. Indeed, some evidence points to reverse transfer of bacteria from the infant during suckling. More research is needed which focusses on the transfer of bacteria between mother and infant during breastfeeding and how this influences infant gut colonisation and the microbiota maturation process. This would allow a greater understanding of the impact of maternal diet, body composition, use of probiotics and other factors which could enable more healthful transfer to occur.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/BM2021.0098.

Table S1. Details on study design, sample size, sample collection details and maternal confounders for high and medium scoring papers for bacterial analysis considered for breast milk composition or for transfer of microbiota to the infant.

Table 2. Details on sample handling and sample analysis for high and medium scoring papers for bacterial analysis, considered for breastmilk composition or for transfer of microbiota from breastmilk to the infant.

Table S3. Studies which consider similarity between milk and infant faeces and potential transfer to infant.

Table S4. List of papers scored for inclusion criteria in the systematic review, but not selected for final assessment based on a low score for bacterial analysis and/or low scores for study design or sample collection details.

Table S5. Details on study design, sample size, sample collection details and maternal confounders for low scoring papers not considered for breast milk composition.

Table S6. Details on sample handling and sample analysis for low scoring papers not considered for breastmilk composition.

Author contributions

CAE, CvLB, JvD, MS, SO, CS, VM, RR, AG, EvdB conceptualised, conducted the research, EvdB, SO, MS, CvLB, CAE, JvD, CS screened papers, AG, EvdB, SO, CvLB, CAE, JvD evaluated methods and scored for milk, bacterial methods, and for transfer data, CAE, CvLB, JvD, RR, AG, EvdB drafted initial subsections of the manuscript, with further input from KV and MF, CAE, CvLB, JvD, RR, AG, EvdB: finalised the manuscript; and all authors read and approved the final manuscript.

Conflict of interest

This work was conducted by an expert group of ILSI Europe. This publication was coordinated by the Early Nutrition and Long-Term Health Task Force. Industry members of this task force are listed on the ILSI Europe website at http://ilsi.eu/task-forces/nutrition/early-nutrition-and-long-term-health/. Experts are not paid for the time spent on this work; however, the non-industry members within the expert group were offered support for travel and accommodation costs from the Early Nutrition and Long-Term Task Forces to attend meetings to discuss the manuscript and a small compensatory sum (honoraria) with the option to decline. The expert group carried out the work, i.e. collecting/analysing data/information and writing the scientific paper separate to other activities of the task forces. The research reported is the result of a scientific evaluation in line with ILSI Europe's framework to provide a precompetitive setting for public-private partnership (PPP). ILSI Europe facilitated scientific meetings and coordinated the overall project management and administrative tasks relating to the completion of this work. For further information about ILSI Europe, please email info@ilsieurope.be or call +32 2 771 00 14. The opinions expressed herein and the conclusions of this publication are those of the authors and do not necessarily represent the views of ILSI Europe nor those of its member companies.

CvLB is an employee of Yili Innovation Center Europe, JvD is an employee of Reckitt|Mead Johnson Nutrition Institute, RR is an employee of Abbott Nutrition, EvdB is a former employee of Danone Nutricia Research. Other authors have no competing interests.

Data availability

The authors confirm that most data supporting the findings of this study are available within the article and its supplementary materials. Additional data supporting this systematic review are available from the corresponding author, or CE, upon reasonable request.

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