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Systematic Review of the Effect of Enteral Feeding on Gut Microbiota in Preterm Infants

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Abstract

Objective—To examine the effect of feeding type on microbial patterns among preterm infants and to identify feeding factors that promote the colonization of beneficial bacteria.

Data Sources—PubMed, Cochrane Database of Systematic Reviews, Scopus and CINAHL were thoroughly searched for papers published between January, 2000 and January, 2017 using the keywords *gut microbiome*, *gut microbiota*, *enteral microbiome*, *enteral microbiota*, *premature infant*, *preterm infant*, *extremely low birth weight infant*, *ELBW infant*, *very low birth weight infant*, *feeding*, *breast milk*, *breastfeeding*, *formula*, *prebiotic*, *probiotic*, and *long chain polyunsaturated fatty acid*.

Study Selection—Primary studies written in English and focused on the association between enteral feeding and gut microbiome patterns of preterm infants were included in the review.

Data Extraction—We independently reviewed the selected articles and extracted information using predefined data extraction criteria including study design, study subjects, type of feeding, type and frequency of bio-specimen, microbiology analysis method and major results.

Data Synthesis—In four of the 18 studies included in the review, researchers described the effects of milk products (mother's own milk, donor human milk, and formula). In five studies, prebiotics were assessed, and in nine studies, the effect of probiotics on gut microbiome was described. Mother's own breastmilk feeding influenced the compositional structure of preterm infants' gut microbial community and increased diversity of gut microbiota compared to donor human milk and formula feeding. The results of the use of prebiotics and probiotics varied among studies; however, the majority of the researchers reported positive bifidogenic effects on the development of beneficial bacteria.

Conclusion—Mother's own milk is considered the best form of nutrition for preterm infants and the gut microbial community. Variation in fatty acid composition across infant feeding types can

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affect microbial composition. The evidence for supplementation of prebiotics and probiotics to promote gut microbial community structure is compelling, however, additional research is needed in this area.

Keywords

Feeding; Mother's own milk; donor human milk; formula; prebiotics; probiotics; long chain polyunsaturated fatty acids; microbiome; systematic review

Human gut microbiota refers to all microorganisms that reside in the digestive tracts of humans, including bacteria, viruses, fungi, and other microbes in which bacteria comprise the most of the flora (Guarner & Malagelada, 2003). The microbiome refers to the genomic elements of the whole microbiota (D'Argenio & Salvatore, 2015). The gut microbial community benefits humans in many different ways, including the fermentation of indigestible dietary fiber into short-chain fatty acids (SCFAs) and provide essential chemical compounds like hormones, essential fatty acids and vitamins to maintain proper function of the human body. Gut microbiota also have important roles in modifying host immune function and inflammatory responses to resist pathogenic organisms, and in regulating the development of the gut defense systems (Boleij & Tjalsma, 2012; Quigley, 2013; Round & Mazmanian, 2009).

Our knowledge of bacteria communities continues to broaden along with culture-independent molecular techniques used to detect an expansive microbial world. Measures commonly used to evaluate gut microbiome include microbial alpha-diversity (α -diversity), beta-diversity (β -diversity), abundance and incidence of individual species (Lozupone & Knight, 2008; Morgan & Huttenhower, 2012). The α -diversity estimates the richness and evenness of the bacterial species in a community (Morgan & Huttenhower, 2012). In most circumstances, a high α -diversity indicates a healthier and more mature microbiome pattern and is often quantified by Simpson Diversity Index and Shannon Diversity Index in human gut microbiome studies. The β -diversity measures the number or the presence/absence of species shared among microbial communities, and evaluates the extent to which two or more communities differ among each other (Lozupone & Knight, 2008). β -diversity is also used to evaluate the changes of gut microbial community over time, i.e., due to environmental or disease status and can be quantified by the Bray-Curtis Dissimilarity index (Kapiki et al., 2007).

Abundance and incidence are used to evaluate individual bacterial species. Abundance of a bacteria species refers to the number of individual species found in a community, whereas incidence measures the frequency of each bacterial species found in communities. In general, high abundance of beneficial microbiota, i.e., *Bifidobacterium* and *Lactobacillus* are more desirable, whereas pathogenic bacteria, including *E. coli*, *Enterobacter*, *Citrobacter*, *Proteus*, *Klebsiella*, and *Candida* are less desirable in the promotion of gut health among preterm infants.

The alteration or imbalance of microbiota, also named dysbiosis or dysbacteriosis, can disrupt human wellness, particularly among vulnerable infants born prematurely (<37 weeks gestational age). Dysbacteriosis was associated with increased risk of colic (Kianifar et al.,

2014) and necrotizing enterocolitis (NEC) in preterm infants (Thomas, 2016; Warner et al., 2016) and can lead to immune disorders (e.g., inflammatory bowel disease; Burcelin, 2016; Jiang et al., 2015; Marteau, 2009), diabetes (Rozanova, Voevodin, Stenina, & Kushnareva, 2002), obesity (Menni et al., 2017; Turnbaugh et al., 2009), and cancer (Loo et al., 2017; Yamamoto & Matsumoto, 2016; Zhu, Gao, Wu, & Qin, 2013) later in life. Preterm infants, especially very low birth weight (VLBW) infants, are susceptible to imbalanced gut microbial community due to gut immaturity (Groer et al., 2014). Other environmental factors that influence the development of a normal gut symbiosis in preterm infants may include perinatal/postnatal use of medication (i.e., intensive use of antibiotics), host genetic factors, mode of delivery, NICU environments, stressful early life events, mother-infant contacts, feeding compositional differences and practices during the perinatal period (Cong et al., 2016).

Total fat in infant feedings comprises 40% of calories ingested. Evidence from human and animal investigations suggests that dietary fatty acid composition likely plays a role in gut microbiota development and composition (Balfegó et al., 2016; Ghosh, Molcan, DeCoffe, Dai, & Gibson, 2013; Noriega, Sanchez-Gonzalez, Salyakina, & Coffman, 2016; Pu, Khazanehei, Jones, & Khafipour, 2016). Given this potential, it is important to consider that fatty acid composition and bioavailability differs among feedings provided to preterm infants based upon the type of feeding they are provided. Although the percent of total calories from fat is similar between human milk and infant formula, the composition of specific fatty acids can vary widely based upon maternal factors (i.e. maternal diet, stage of lactation) or compositional differences between formula type provided (i.e. standard preterm, elemental) (Robinson & Caplan, 2015). These compositional differences affect percent and form of saturated, monounsaturated, and long chain polyunsaturated fatty acids provided to infants with implications for microbiota development and associated health outcomes.

Prebiotics are the non-digestible food ingredients that stimulate the growth and/or activity of beneficial bacterial species (e.g., Bifidobacteria) and thereby potentially improve the host health (Gibson & Roberfroid, 1995). The human alimentary enzymes are not able to digest most complex carbohydrates and plant polysaccharides. Instead, these polysaccharides are metabolized by microbes which generate short-chain fatty acids, including acetate, propionate, and butyrate (Holscher, 2017). One of the well-known beneficial prebiotics for preterm infants are oligosaccharides contained in human milk (human milk oligosaccharides, HMOs), which has been shown to facilitate the growth of *Bifidobacteria* and *Lactobacillus* (Dai & Walker, 1999). With regard to oligosaccharides, it is important to highlight that oligosaccharides are naturally abundant in human milk and are highly complex and structurally different from those added to infant formulas (Ninonuevo & Bode, 2008). Over 200 HMOs have been found in human milk (Bier, German, & Lönnerdal, 2008; German, Freeman, Lebrilla, & Mills, 2008; Ninonuevo et al, 2006). The HMOs are not only complex in structure but vary from mother to mother depending on her Lewis blood group and secretor status (Rudloff & Kunz, 2012). Given the high composition of HMO in human milk and associated health benefits, formula companies have attempted to find inexpensive alternatives including galactooligosaccharides (GOS), and fructooligosaccharides (FOS) to mimic HMO. Galactooligosaccharides and FOS have similar bifidogenic effects (ability to

promote bifidobacterial growth) compared to HMOs (Boehm et al., 2002; Kapiki et al., 2007). A structural difference with clinical significance relates to immunogenic properties of HMO. Although HMOs structurally resemble epithelial cell surface glycans and block pathogen adhesion, structural differences in GOS and FOS may not yield the same protection from diarrhea and other infections (Newburg, Ruiz-Palacios, & Morrow, 2005). Further research is necessary related to the potential benefits of GOS and FOS in infant formulas given structural differences to further demonstrate related health outcomes and the cost effectiveness of large-scale use. Additionally, prebiotics are of specific research interest in relation to their promotion of microbes that produce short chain fatty acids that help with optimal functionality of the gut.

In contrast to prebiotics, probiotics refer to live microorganisms that are administered to the human body in order to attain optimal health. The commonly used probiotics are *Lactobacillus* and *Bifidobacterium* species in infants. The benefits related to probiotic use include reduced pathogenic bacteria, increase of beneficial bacteria, improvement of food tolerance, and decrease of incidence of nosocomial sepsis and NEC in preterm infants (Al-Hosni et al., 2012; Braga, da Silva, de Lira, & de Carvalho, 2011; Luedtke, Yang & Wild, 2012).

Feeding has been recognized as one of the most influential factors contributing to the early development of the gut microbiome because milk is the first food to be introduced to the digestive tract in infancy (Cong et al., 2017; Gregory et al., 2016). In addition, prebiotics and probiotics are often supplemented with milk in order to increase the colonization of beneficial bacteria and to promote the maturation of the gut community. However, the effects of different feeding regimens on gut microbiota patterns remain unclear. The purpose of this systematic review was twofold: to describe the patterns of microbial community of infants with different feeding type and to identify the optimal feeding types in promoting the colonization of beneficial bacteria among preterm infants.

Methods

Eligibility Criteria of the Literature

This systematic review was conducted following the guidelines in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement for reporting systematic reviews (Liberati et al., 2009). Original research studies on the effect of enteral feeding types on gut microbiological composition and diversity were included. Articles were written in English and published after January, 2000. No restrictions on study design were imposed.

We targeted preterm infants who were born at or less than 37 weeks gestational age (GA), were recruited after birth to 30 days of postnatal age, and were followed up for 2 weeks or longer. Studies of preterm infants with congenital anomalies, severe periventricular/intraventricular hemorrhage, who underwent major surgery, or had histories of positive drug exposure were excluded. The primary outcome measures were gut microbiome patterns, including the α -diversity, β -diversity, and abundance of specific bacteria of the gut microbiome. Predicting variables included enteral feeding types, mother's own milk

(MOM), donor human milk (DHM), and infant formula, and dose and frequency of use of prebiotics and/or probiotics. Studies involving in vitro fecal batch culture or gut microbiome of animals were excluded from the review.

Information Sources and Search Strategies

Research databases were systemically searched to identify eligible studies, including PubMed, Cochrane Database of Systematic Reviews, Scopus, and CINAHL. Keywords included *gut microbiome*, *gut microbiota*, *enteral microbiome*, *enteral microbiota*, *premature infant*, *preterm infant*, *extremely low birth weight infant*, *ELBW infant*, *very low birth weight infant*, *feeding*, *breast milk*, *breastfeeding*, and *formula*. The last search was run on January 31st, 2017. In addition, we hand-searched the reference pages of the reviewed studies.

Study Selection

The studies were screened by two reviewers independently in an un-blinded standardized manner based on the eligibility criteria. Disagreements between reviewers was resolved by mutual agreement. If agreement was not achieved, a third reviewer was involved for a final decision.

Data Extraction

All selected articles were independently reviewed by two researchers using a predefined data extraction form that was modified based on Cochrane Consumers and Communication Reviews Group's data extraction template and adapted for use in the assessment of non-randomized studies. Information including author, country, year, study design, study subjects, type of feeding, type and frequency of bio-specimen, microbiology analysis method and major results were collected by each researcher independently and consensus between two reviewers was achieved by discussion.

Risk of Bias

The internal validity of each individual study was evaluated using Grading, Recommendations, Assessment, Development and Evaluation (GRADE) guidelines (Balslem et al., 2011). According to GRADE rating approach, randomized control trials (RCTs) were rated as high-quality evidence, and reviewers needed to consider features such as randomization, allocation concealment, blinding and completeness of the outcome data. In contrast, observational studies were rated as low-quality evidence and evaluated by appropriate measurement of exposure and outcome, effective control of confounding, and the extent of loss to follow-up.

Data Analysis

The evidence on gut microbiota outcomes were grouped according to feeding regimens, including milk category (MOM, DHM, and infant formula) and use of prebiotics and/or probiotics. Studies on the same bacterial species were analyzed by grouping together. Microbial α -diversity (the richness and/or evenness of a microbiome community) and β -

diversity (the compositional dissimilarity among the microbial community) measured using the same method were also grouped and analyzed together under the same category.

Results

Selection of the Literature

The search yielded a total of 136 articles. The titles and abstracts were reviewed to exclude the articles that were not related to the research questions. One hundred and seven articles were excluded after the first screening, including 92 articles that were not relevant and 11 duplicates. The remaining 33 research articles were evaluated by review of the full-text, and 12 were further excluded because the authors used in vitro study design and they did not meet inclusion criteria. Meanwhile, three pairs of articles were identified as duplicates of the same study in different journals; we selected the one of each pair that reported the full dataset. In total, 18 articles were identified as eligible for the final systematic review. No doctoral dissertations or unpublished articles were included. See Figure 1, the PRISMA flow diagram, for details related to the search strategy.

Methodologies Used in the Included Studies

In the identified studies, six were conducted in the United States (Cong et al., 2017; Gregory et al., 2016; La Rosa et al., 2014; Patel, Konduru, Patra, Chandel, & Panigrahi, 2015; Underwood et al., 2013; Underwood et al., 2014), two in France (Campeotto et al., 2011; Rouge et al., 2009), two in Germany (Boehm et al., 2002; Mohan et al., 2006), two in Italy (Manzoni et al., 2006; Romeo et al., 2011), one in Iran (Armanian et al., 2016), one in the Netherlands (Westerbeek et al., 2013), one in Greece (Kapiki et al., 2007), one in Spain (Moles et al., 2015), one in Poland (Chrzanowska-Liszewska, Seliga-Siwecka, & Kornacka, 2012), and one in Japan (Li et al., 2004). Two studies were observational (Cong et al., 2017; La Rosa et al., 2014), two were prospective cohort (Gregory et al., 2016; Patel et al., 2015), and one was quasi-experimental without a control group (Moles et al., 2015); the remaining articles were randomized controlled trials (Tables 1–3). In terms of microbiological analyses, in three of the studies the authors used 16sRNA next generation sequencing, in two they used Terminal restriction fragment length poly-morphism (TRFLP), in two they used temporal temperature gel electrophoresis (TTGE), in two they used fluorescent in situ hybridization (FISH), in one they used pulsed-field gel electrophoresis (PFGE), and in 10 they used a culture method. Among these, in four studies authors combined the culture method with other analysis technology (Tables 1–3).

Relation of Feeding Types and Gut Microbiome

Preterm infants who were fed with MOM had higher initial α -diversity of their gut microbial communities (Cong et al., 2017; Gregory et al., 2016). Microbial α -diversity index in infants fed with DHM and formula remained lower during the first 30 days of life (Cong et al., 2017) then increased to a relative higher level at 60 days after birth or 40 weeks adjusted gestational age compared to infants fed with MOM (Gregory et al., 2016). Furthermore, α -diversity in infants fed with DHM and formula had higher variance across subjects compared to the infants fed with MOM, which indicated their susceptibility to the influence of other factors, especially birth gestational age and birth weight (Gregory et al., 2016).

Gut microbial β -diversity measured using the Bray-Curtis Dissimilarity Index indicated that infants fed with MOM clustered together (high similarity) and were separate from infants fed with DHM and formula (Cong et al., 2017; Gregory et al., 2016). Cong et al. (2017) also reported that feeding type explained 11% of the variance in the community composition ($p < 0.001$). In addition, the structure of gut microbial community in infants fed with MOM was less susceptible to the influence of birth weight than infants fed with formula who were reported to have a more scattered plot distribution or dissimilarity by birth weight ($p < 0.01$) (Gregory et al., 2016).

Two groups of researchers reported that infants fed with MOM had higher levels of *Clostridiales* than infants fed in other ways (Cong et al., 2017; Gregory et al., 2016). La Rosa et al. (2014) reported that MOM was associated with increased colonization of *Gammaproteobacteria* ($p < 0.04$). Within this class, the association between the colonization of *Enterbacteriales* with MOM remains controversial across investigations (Cong et al., 2017; Gregory et al., 2016). Gregory et al. (2016) reported that infants fed with formula had delayed development of *Enterbacteriales* compared to infants fed with MOM whereas Cong (2017) reported higher *Enterbacteriales* colonization in infants fed with DHM and formula compared to infants fed with MOM throughout the first 30 days of life. Similarly, a lack of congruence existed across investigations in the association between infants fed with MOM and *Lactobacillales* and *Bacillales* colonization (Cong et al., 2017; Gregory et al., 2016). No difference was reported for the effect of infants fed with MOM and formula and the colonization of *Lactobacillus* (Patel et al., 2015). Interestingly, Cong et al. (2017) reported infants who were fed by a combination of MOM and formula had higher colonization of *Bifidobacteriales*. Similarly, within the order of *Bifidobacteriales*, infants fed with MOM had higher levels of *Bifidobacteria* than infants fed with DHM and formula (Patel et al., 2015). In summary, MOM promoted a rich gut microbial community and supported the development of *Clostridiales* and *Bifidobacteria* colonizations compared to other sources (Table 1).

Relation of Prebiotic Use and Gut Microbiome

Human milk oligosaccharides (HMO) are structurally diverse glycans found primarily in human milk. Numerous variations in structure and concentration of HMOs have been found between individual infants and over the course of lactation (Chaturvedi et al., 2001). Several researchers found that HMO were associated with a protective effect that included the inhibition of adhesion and invasion of pathogenic microorganisms to the intestinal epithelial wall (Gonia et al., 2015; Ruiz-Palacios, Cervantes, Ramos, Chavez-Munguia, & Newburg, 2003), regulation of bacteria-host interactions, and modulation of microbial composition of human gut (Bode, 2009).

Galactooligosaccharides and Fructooligosaccharides are commercial prebiotics with Bifidogenic effects like HMO. Underwood et al. (2014) compared the bifidogenic effects between GOS and HMO in preterm infants fed with formula, and reported no differences in α -diversity or individual species including *Proteobacteria*, *Bacilli*, *Bifidobacteria*, or *Clostridia* over time (Underwood et al., 2014). Kapiki (2007) reported that preterm infants with formula feeding regimens that included a supplement of FOS (0.4 g/100 ml) had

significantly higher numbers of *Bifidobacteria* at day 7 after the infants reached full formula feeding compared to the placebo group. In addition, the FOS supplemented feeding group had lower concentrations of *E. coli*, *Bacteroides*, and *Enterococci* than the non-FOS cohorts (Kapiki et al., 2007).

Three groups of researchers investigated the effect of the GOS/FOS combination on the gut microbial composition and yielded contradictory results (Armanian et al., 2016; Boehm et al., 2002; Westerbeek et al., 2013). Armanian (2016) observed that preterm infants fed with breast milk and supplemented with GOS/FOS (9:1) had significantly higher colony counts of *Lactobacillus* and lower counts of *Coliforms* over the three study time points than infants in the control group. The difference disappeared at the last study point for *Lactobacillus* when the feeding volume reached 110 – 150 ml/kg/day. In contrast, Boehm (2002) reported no difference in the colony counts of *Lactobacilli* between the GOS/FOS (9:1) group and placebo group at any of the 4 study points. In addition, colony counts of potentially pathogenic microorganisms, including *Bacteroides*, *Clostridium* species, *E. coli*, *Enterobacter*, *Citrobacter*, *Proteus*, *Klebsiella*, and *Candida*, were similar in both groups (Boehm et al., 2002). Westerbeek (2007) reported similar results to Boehm et al. and found infants supplemented with a prebiotic mixture with 80% GOS/FOS (9:1) and 20% acidic oligosaccharides (AOS) had higher *Bifidobacteria* counts compared to the control group (Boehm et al., 2002; Westerbeek et al., 2013). The overall results indicated that prebiotics were bifidogenic efficient in stimulating the growth of *Bifidobacteria* (Table 2).

Relation of Probiotic Use and Gut Microbiome

Researchers in nine of the 18 included articles assessed the effect of probiotics on gut bacterial colonization; increased abundance or incidence of *Bifidobacteria* and/or *Lactobacillus* after treatment with a probiotic was found in five studies (Chrzanowska-Liszewska et al., 2012; Li et al., 2004; Mohan et al., 2006; Rouge et al., 2009; Underwood et al., 2013). Li et al. (2004) found that introduction of *Bifidobacteria breve* (*B. breve*) supplementation within 24 hours of life was associated with early colonization of *Bifidobacteria* (3.4 ± 2.2 day) and decreased abundance of *Enterobacteriaceae* two weeks after birth ($p < 0.05$). No statistical difference was reported in the rates or abundance of *Streptococcus* and *Staphylococcus* in association with *B. breve* supplementation (Janvier, Malo, & Barrington, 2014). Similarly, Mohan et al. (2006) conducted a RCT and reported that infants supplemented with *Bifidobacterium lactis* (*B. lactis*) group had a higher abundance of *Bifidobacteria* and lower abundance of *Clostridia* and *Enterobacteriaceae* of gut microbiome compared to the control group. The investigators also reported no difference between the probiotics and control groups in the levels of *Staphylococcus spp.*, *Streptococcus spp.*, *Bacteroides spp.*, and *Candida spp.* Rouge et al. (2009) evaluated the effect of combined supplementation of *Bifidobacterium longum* and *Lactobacillus rhamnosus* on gut microbiota compositions. The incidence of gastrointestinal colonization of *Bifidobacteria* and *Lactobacilli* was significantly higher in the probiotic group than in the control group. Chrzanowska-Liszewska et al. (2012) conducted a double-blind RCT in preterm infants born at <32 weeks gestational age and found that infants fed with formula and supplemented with *L. rhamnosus* during the first 42 days had higher incidence of *Lactobacillus* on day 7 and day 21 postnatal age but not on day 42 than infants in the control group.

In contrast to previous investigator findings, Chrzanowska-Liszewska and colleagues reported that infants in the probiotic group had higher incidence of *Enterobacteriaceae*, *Enterococcus*, and *Staphylococcus* than infants in the control group. In contrast, some investigators found no relationship between use of probiotics and gut microbial development. Campeotto et al. (2011) reported that the combination of heat-killed *B. breve*, *Streptococcus thermophiles* (*S. thermophiles*) strain and non-digestible oligosaccharides added to formula and fed to preterm infants did not alter bacterial colonization, including *Bifidobacteria*, *Lactobacilli*, *Escherichia coli*, *Klebsiella*, and *Enterobacter cloacae*. In a one-group pretest-posttest study with 5 preterm infants born at less than 29 weeks gestational age, Moles (2015) reported that the supplementation of *B. breve*/*L. salivarius* had no modifying effect on the colonization of *Bifidobacteria*, *Lactobacillus*, and α -diversity of the microbial community, although *Enterococcus* decreased overtime after introduction of the probiotics.

To investigate the effects of two strains of probiotics on gut microbial community in preterm infants, Underwood et al. (2013) conducted two trials and found that α -diversity increased over time and with the escalation of dose among infants fed with formula and supplemented with *B. infantis* compared to infants fed with formula and supplemented with *B. lactis*. The relative abundance of *Bifidobacteria* was higher in the *B. infantis* group compared to the cohort receiving *B. lactis*. In addition, early administration of *B. infantis* was associated with increased abundance of *Bifidobacteria* and decreased *Proteobacteria* in the intestinal tract (Underwood et al., 2013).

Three groups of investigators examined the effect of probiotics on fungal colonization, and two reported reduction of fungi after a treatment with probiotics. Manzoni et al. (2006) implemented a 6-week, double-blind RCT in low birth weight, preterm infants and found that *L. rhamnosus* supplementation with breast milk (MOM or DHM) was associated with a significant reduction in the number of fungal isolates per infant and incidence of fungal colonization, particularly in infants who weighed more than 1000g at birth. Romeo et al. (2011) compared the effect of *L. reuteri* and *L. rhamnosus* and also found that both probiotic strains lowered the incidence of gut fungal colonization. In contrast, Mohan et al. (2006) found that consumption of the *B. Lactis* supplement did not reduce the abundance of *Candida* in the gut of preterm infants fed with formula. Overall, results related to probiotic use and bacterial species in preterm infants' gut communities were contradictory. Nevertheless, the majority of the investigators supported the positive effect of probiotics on the growth of *Bifidobacterium* and the reduction of fungal colonization (Table 3).

Discussion

Our primary findings include the following: (a) MOM influences the compositional structure of preterm infants' gut microbial community and increases diversity of gut microbiota compared to DHM and formula; (b) Breast milk contains complex oligosaccharides, lactoferrin, and beneficial bacteria that stimulate the growth of specific bacterial groups such as *Clostridiales* and *Bifidobacteria*; (c) Supplementation of commercial prebiotics, particularly GOS and FOS, promotes the development of *Bifidobacteria* colonization; and

(d) Supplementation of probiotics may increase the colonization of beneficial bacteria, particularly *Bifidobacterium* and *Lactobacilli*, and inhibit fungal colonization.

Mother's own milk is effective in improving gut health by increasing the diversity of gut microbial community and promoting the maturation of gut microbiota and early transition to a more adult-like microbial composition. Although the existing evidence regarding feeding and microbiota development in preterm infants focuses on type of milk provided, recent evidence supports the need for more careful consideration of the specific composition which varies across milk types. Given a significant contribution of dietary fat, 40% of total calories, evidence from human and animal investigations in other populations support an interaction between specific dietary fatty acid components and gut microbiota composition (Balfegó et al., 2016; Ghosh et al., 2013; Noriega et al., 2016; Pu et al., 2016). Maternal factors that include diet, stage of lactation, and term or preterm birth can affect the fatty acid composition of human milk. Additionally, the fat blend and composition of specific fatty acids in infant formulas varies between manufacturers and between products produced by the same manufacturer dependent upon the formula used and clinical necessity (Robinson & Caplan, 2015).

Although we identified a gap in the literature related to fatty acid composition and microbiota composition in preterm infants, the results of several recent investigations demonstrate that dietary long chain polyunsaturated fatty acid composition significantly affects gut microbial composition (Balfegó et al., 2016; Ghosh et al., 2013; Noriega et al., 2016; Pu et al., 2016). The long chain omega-3 fatty acid (LCPUFA) docosahexaenoic acid (DHA), an anti-inflammatory fatty acid, beneficially altered microbiota composition in human (Balfegó et al., 2016; Pu et al., 2016) and mouse models (Yu et al., 2014). These microbial benefits related to omega-3 LCPUFA have been associated with increased composition of butyrate-producing bacteria (Noriega et al., 2016). The short chain fatty acid butyrate has been associated with optimal functional microbiota function and associated outcomes (Holscher, 2017). In contrast, in an animal model, high pro-inflammatory omega-6 LCPUFA was associated with dysbiosis of gut microbiota (Ghosh, Molcan, DeCoffe, Dai, & Gibson, 2013). Collectively, these findings support a role of high omega-3 LCPUFA in modulating the gut environment and influencing better microbiota composition. Further work is necessary regarding the evaluation of the contribution of specific dietary components, including associations among omega-3 LCPUFA, microbial diversity, and type (Robinson & Caplan, 2015).

Donor human milk, although a preferable alternative for preterm infants, is less effective to promote the growth of beneficial bacteria and the diversity of the entire gut community than MOM (Cong et al., 2017; Gregory et al., 2016). This may be explained by the pasteurization process and destruction of the immunological, nutritional, and microbial components in DHM that impair the interaction of the microbiome and oligosaccharides (Landers & Hartmann, 2013; Neu, 2015; Reeves, Johnson, Vasquez, Maheshwari, & Blanco, 2013). Interestingly, evidence suggests that formula may be effective to increase beneficial bacteria, particularly *Lactobacillales* (Gregory et al., 2016) and *Bifidobacteriales* (Cong et al., 2017). Since the ingredients and the manufacturers of the formulas used in each study were not reported, the evidence is unclear regarding how preterm infant formula could promote the

growth of certain bacteria. One possible explanation is that some formula has been fortified with probiotics and/or prebiotics that support the proliferation of *Bifidobacteria* and *Lactobacillus*.

Prebiotic is a non-digestible food ingredient that promotes the growth and/or activity of beneficial microorganisms. Prebiotics pass through the upper gastrointestinal systems unabsorbed and selectively stimulate the intestinal bacteria especially *Bifidobacteria* and lactic acid bacteria that are associated with health (Gibson, Probert, Loo, Rastall, & Roberfroid, 2004). The findings of this review showed that commercial oligosaccharides, specifically GOS and FOS, are effective to promote the colonization of *Bifidobacteria* in the digestive tracts of preterm infants although their overall effect on intestinal health remains to be studied. The result is consistent with the conclusion from Srinivasjois, Roa & Patoles systematic review in 2013. There may be undesirable side effect related to commercially available prebiotics. An animal study indicates supplementation of GOS/inulin increase bacterial translocation in newborn rats (Barrat et al, 2008). However, no study has reported bacterial translocation among preterm infants. Notably, the studies included in the review reported that supplementation of prebiotics to preterm infants was safe and well-tolerated without side effects such as diarrhea, food intolerance, fluid imbalance, abdominal distention, vomiting or weight loss (Armanian et al., 2016; Boehm et al., 2002; Kapiki et al., 2007; Underwood et al., 2014; Westerbeek et al., 2013). Therefore, more investigations are needed to evaluate the efficacy and safety of prebiotics use among preterm infants.

Probiotics refer to live microorganisms that provide health benefit to the host when administered in adequate dose (Hill et al., 2014). It is well known that probiotics are associated with the inhibition of pathogenic bacteria, normalization of perturbed microbiota and restoration of healthy microbiota community (Reid et al., 2011). Previous researchers found that administration of probiotics in preterm infants was associated with increased feeding tolerance (Aceti et al., 2016; Whelan, 2007; Whelan, Gibson, Judd, & Taylor, 2001), reduced incidence of necrotizing enterocolitis and sepsis (AlFaleh & Anabrees, 2014; Deshpande, Rao, & Patole, 2007; Deshpande, Rao, Patole, & Bulsara, 2010; Fernandez-Carrocera et al., 2013; Guthmann et al., 2010; Janvier et al., 2014; Mihatsch et al., 2012; Wang, Dong, & Zhu, 2012), and better neurological and immunological outcomes (Romeo et al., 2011). Researchers also demonstrated the safety of enteric administration of probiotics, especially certain strains of *Bifidobacteria* and *Lactobacillus* in preterm infants. Nevertheless, the results of this review highlight controversial effects of probiotics on the microbiome patterns. Several articles in this review included non-significant bifidogenic effects of probiotics with preterm infants. The reasons are multifactorial and may be related to the efficacy of specific manufacturing processes, doses and frequency of use related to each probiotic strain, analytical issues, or the combination of these factors. Despite discrepancies, the majority of the studies demonstrated positive effects of probiotics on the development of preterm infants' gut microbiota.

Strengths and Limitations of the Review Method

We used PRISMA as reporting guideline to ensure quality. Furthermore, we used GRADE to examine the quality of the evidence from original studies and to minimize the risk of bias.

Limitations of the review process also need to be considered. In several of the included studies, design and interventions were not thoroughly described. Moreover, the dose/frequency of intervention and the outcome measurements of gut microbiome community differed among studies, which impeded the synthesis of the findings. Lastly, we only included articles published in English, which may limit the credibility and generalizability of the findings.

Strengths and Limitations of the Evidence from the Review

It is still a challenge to investigate gut microbiome and infant feeding because there are no standard measures with which to evaluate and analyze microbial taxa. Rapid advances in microbial community sequencing have now made it possible to conduct large scale studies to compare the effects of preterm infants fed with MOM, DHM and formula on gut microbial development; however, to date few have been reported. Findings from the studies synthesized in this review revealed an inconsistent relationship between feeding types and gut microbiome patterns in preterm infants. Agreement on observed colonization pattern of some specific bacteria across different studies was challenging and may be due to differences in microbiome isolation, sequencing and analysis techniques, or the limited size of studies published to date. A recent review highlights that earlier culture-dependent methodologies, although useful in the isolation of specific bacterial strains, do not adequately identify the pattern of the microbial community due to the absence of account for the uncultivable microorganisms (Arnold, Roach, & Azcarate-Peril, 2016). Arnold et al. (2016) used 16sRNA isolation combined with denaturing gradient gel electrophoresis (DGGE), pulsed-field gel electrophoresis (PFGE), terminal restriction fragment length polymorphism (TRFLP), or temporal temperature gel electrophoresis (TTGE) method to detect the differences between microbial communities. However, these techniques were not able to generate taxonomic structures of microbial communities. Only three groups of researchers used DNA sequencing analysis of the 16S rRNA gene using next generation sequencing. Although this technique provides richer information of complex microbial community, it has only recently become cost effective. Target gene sequencing has several potential biases due to its reliance on PCR to generate material for sequencing including primer efficiency, PCR amplification conditions, sequences platform, bioinformatics pipeline, and protocols for DNA extraction (Arnold et al., 2016; Huse, Ye, Zhou, & Fodor, 2012).

In addition to the technical issues associated with data analysis, other factors that led to the variation of results across investigations included differences in the gestational age of study population, mode of delivery, different enteral feeding practices, manufacture of the preterm infant formula, prebiotics, and probiotics, medication use especially antibiotic use protocols. Moreover, NICU's environmental influence, such as environmental microbial composition and differences in hygiene protocols for health care providers and visitors may also bias the microbiome outcome across the studies.

Conclusion

Mother's own milk has is the most beneficial form of nutrition for preterm infants and their gut microbial community. As health care providers, we need to discuss the benefits of breast milk and encourage breastfeeding among mothers who give birth to preterm infants. Furthermore, nutritional education for breastfeeding mothers to support optimal breast milk composition may have implications for early microbiota establishment. When MOM is not available, supplementation of prebiotics and/or probiotics to DHM or formula is a plausible option for optimizing the microbial community structure, but the definitive data on dose and efficacy of these products has not been determined.

Although multiple investigators s evaluated the effect of feeding type on preterm infant's gut microbiome, most focused on feeding type and have not considered the potential influence of individual nutritional components that include LCPUFA. Investigation of the functionality of the gut microbiome in preterm infants and production of short chain fatty acids could further our understanding of the role of gut microbes and associated function in this specific population.

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Callouts

1. The purpose of this systematic review was to examine the association between feeding types and the development of the gut microbiome in preterm infants.
2. Mother's own milk is the best type of nutrition to promote the maturation of gut microbiota that resemble a more adult-like microbial structure.
3. Supplementation of prebiotics and/or probiotics may promote development of beneficial microorganisms, particularly microbes that generate short chain fatty acids and *Bifidobacterium* colonization in preterm infants.

Figure 1.
Review of enteral feeding and gut microbiome in preterm infants. Flow chart based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.

Table 1

Studies on Feeding Types and Gut Microbiome Outcomes

First Author/Region/Year	Study Design	Study Subjects	Type of Feeding	Bio-specimen	Microbiology Analysis	Major Result
Cong/ USA /2017	Observational longitudinal study	N = 33; 28–32 weeks BGA	MOM, DHM, formula	419 daily feces; birth to 30 days	16S rRNA genes (V4 region), MiSeq, α -diversity, β -diversity, bacterial taxonomical composition	MOM-fed infants had highest abundance of <i>Clostridiales</i> , <i>Lactobacillales</i> , and <i>Bacillales</i> ; DHM or formula-fed infants had a high abundance of <i>Enterobacteriales</i> . α -diversity of gut microbiome increased over time and was constantly higher in MOM-fed infants relative to infants with other feeding types ($p < 0.01$).
Gregory/ USA /2016	Prospective cohort study	N = 30; < 32 weeks BGA	MOM ($n = 10$); DHM ($n = 10$); formula ($n = 10$)	199 daily feces, birth to 60 days	16S rRNA genes (V4 – V5 region), MiSeq, α -diversity, β -diversity, bacterial taxonomical composition	Intestinal microbiome was influenced by nutrition ($p < 0.001$). α -diversity in MOM-fed infants was higher and increased gradually compared to formula-fed infants. The microbiome clustered together for breastmilk-fed infants ($p = 0.049$) but separately based on birth weight for formula-fed infants ($p < 0.001$). Microbiomes of DHM-fed infants were more similar to breast milk-fed infants.
Patel/ USA/ 2015	Prospective cohort study	N = 22; 24–32 weeks BGA; < 1500g BW	Exclusively breast milk ($n = 11$); breast milk and formula ($n = 11$)	Weekly gastric aspirate; birth to 4 weeks	16S rRNA (V3 region), PCR, DGGE, microbial composition	<i>Bifidobacterium</i> was higher in exclusively breast milk-fed compared to partially breast milk-fed neonates in the first ($p = 0.03$) and third ($p = 0.03$) weeks of life. Anaerobic bacteria colonization decreased from first through fourth week of life ($p = 0.03$). Birth weight was positively correlated with total number of bacterial species ($p = 0.002$) and anaerobes ($p = 0.004$) in PBM-fed neonates during the fourth week of life.
La Rosa/ USA/ 2014	Observational study	N = 58; 1500g BW	Breast milk use	922 feces	16S rRNA gene (V3 - V5 region), Roche-454 platform, bacterial taxonomical composition	<i>Gammaproteobacteria</i> was higher in MOM group. ($p < 0.05$)

Note. RCT = randomized controlled trial; MOM= mother's own milk; DHM= donor human milk; BGA= birth gestational age; BW= birth weight; PCR=polymerase chain reaction; DGGE= denaturing gradient gel electrophoresis.

Table 2

Studies of Prebiotics Use and Gut Microbiome Outcomes

First Author/Region/Year	Study Design	Study Subjects	Type of Feeding	Bio-specimen	Microbiology Analysis	Major Result
Armanian/ Iran/ 2016	Double-center RCT	N = 50; < 1500g BW	Study group: (n = 25) breastmilk + GOS/FOS (9:1); control group: (n = 25) breastmilk	Feces; 1st day and last day of each prebiotic concentration	Culture method, colony counts of <i>Coliforms</i> and <i>Lactobacillus</i>	<i>Coliforms</i> were significantly lower in the third stool cultures in the study group (p < 0.001). <i>Lactobacillus</i> colony counts were higher in the control group for the first stool cultures, (p = 0.005); and a trend toward significance increased in the study group during the study. The difference between <i>Lactobacillus</i> colony counts in the third stool cultures between two groups was not statistically significant (p = 0.11).
Underwood/ USA/ 2014	RCT	N = 27; < 33 weeks BGA; < 1500g BW	Study 1: Formula + HMO group (n = 6); formula + GOS group (n = 6). Study 2: MOM + HMO containing fortifier group (n = 8); MOM + non-HMO fortifier (n = 7)	Study 1: feces at baseline and weekly sample for 5 weeks. Study 2: feces at baseline and every 2 weeks for 6 weeks	TRFLP, α-diversity, bacterial composition	Infants from the Formula + GOS and Formula + HMO groups demonstrated an increase in relative numbers of <i>Clostridia</i> with increasing doses. Compared with the MOM + non-HMO fortifier group, the infants in the Formula+ HMO and the MOM + HMO groups showed an unexpected trend toward an increase in <i>Proteobacteria</i> over time/dose. Principal coordinate analyses and Shannon diversity scores were not significantly different among
Westerbeek/ The Netherlands/ 2013	RCT	N = 113; < 32 weeks BGA; < 1500g BW	Study group (n = 55): 80 % GOS/FOS (9:1) and 20 % AOS; placebo group (n = 58): maltodextrin	Feces at baseline and day 7, 14 and 30 of life.	FISH, bacterial composition	Prebiotic mixture increased the total bacteria count at day 14 (p = 0.03), but not at day 30 (p = 0.31). There was a trend of increased <i>Bifidobacteria</i> counts in study group. The colonization of specific bacterial groups didn't differ between two groups.
Kapiki/ Greece/ 2007	RCT	N = 56; < 36 weeks BGA	Study group (n = 36): formula + FOS (0.4 g/100 ml); placebo group (n = 20): formula + maltodextrins (0.4g/dl)	Feces at day 1 and day 7 of the full formula feeding	Culture method, colony counts	The abundance of <i>Bifidobacteria</i> (p=0.032), the incidence of <i>Bifidobacteria</i> colonization (p = 0.030) and the abundance of <i>Bacteroids</i> (p = 0.029) were significantly high in study group compared to the placebo. The abundance of <i>Escherichia coli</i> and <i>Enterococci</i> was lower in study group. (p = 0.029, and p = 0.025, respectively).

First Author/Region/Year	Study Design	Study Subjects	Type of Feeding	Bio-specimen	Microbiology Analysis	Major Result
Boehm/ Germany/ 2002	RCT	N = 30; < 32 weeks BGA	Study group (n = 15): formula + GOS/FOS (9:1; 10 g/l); placebo group (n = 15): formula + maltodextrin	Feces at day 1, 7, 14, and 28 of the full formula feeding	Culture method, colony counts	Counts of <i>Bifidobacteria</i> increased in both groups. The number of <i>Bifidobacteria</i> in the study group was higher than control groups at 28 days of the study (p = 0.0008).

Note. RCT = randomized controlled trial; MOM= mother's own milk; BGA= birth gestational age; BW= birth weight; HMO= human milk oligosaccharides; GOS= galactooligosaccharides; FOS= fructooligosaccharide; AOS= acidic oligosaccharides; TRFLP= terminal restriction fragment length poly-morphism; FISH= fluorescent in situ hybridization.

Table 3

Studies on Probiotic Use and Gut Microbiome Outcomes

First Author/Region/Year	Study Design	Study Subjects	Type of Feeding	Bio-specimen	Microbiology Analysis	Major Result
Moles/ Spain/ 2015	Quasi-experimenta I study	N = 5; < 29 weeks BGA, < 1300 g BW	<i>B. breve</i> , <i>L. salivarius</i>	5 Meconium at baseline, 14 weekly feces and 10 weekly blood samples	PFGE, abundance of microorganisms	Bacterial growth was detected in all the fecal samples. The phylum <i>Firmicutes</i> dominated in nearly all fecal samples. <i>Bifidobacteria</i> , <i>Lactobacillus</i> and α -diversity didn't differ after intervention.
Underwood/ USA /2013	Study 1: Dose-Escalation trial; Study 2: cross-over trial	N = 21 < 33 weeks BGA, < 1500g BW	Study 1: formula + <i>B. infantis</i> (n = 6); formula + <i>B. Lactis</i> (n = 6). Study 2: MOM + <i>B. infantis</i> / <i>B. Lactis</i> (n = 4); MOM + <i>B. Lactis</i> / <i>B. infantis</i> (n = 5)	Feces at baseline and weekly for 5 weeks	TRFLP, qPCR, microbial composition, α -diversity	<i>Bifidobacteria</i> was higher in formula + <i>B. infantis</i> group than formula + <i>B. Lactis</i> group at a dose of 1.4×10^9 colony-forming units twice daily (p < 0.05). Bacterial diversity improved over dose/time in formula + <i>B. infantis</i> . Human milk-fed infants supplemented with <i>B. infantis</i> / <i>B. Lactis</i> had increased <i>Bifidobacteria</i> and decreased <i>gamma-Proteobacteria</i> than their counterpart.
Chrzanowska-Liszewska / Poland/ 2012	Double blinded RCT	N = 47; < 32 weeks BGA; > 1000 g BW	Study group (n = 21) formula + <i>L. rhamnosus</i> ; control group: (n = 26) formula + maltodextrine for the first 42 days	Feces at day 7, 21 and 42	Culture method, the abundance and prevalence of stool microorganisms	Study group had higher abundance of <i>Lactobacillus</i> (p = 0.014) on day 7, and 21 (p = 0.024), <i>Enterobacteriaceae</i> on all study days (p = 0.004, p = 0.000, p = 0.000), and <i>Enterococcus sp.</i> on day 21 (p = 0.000). The number of samples positive for <i>Staphylococci</i> was significantly higher in the study group, on days 7 and 42 (p = 0.001 and 0.011).
Campeotto/ France/ 2011	RCT	N = 58; < 35 weeks BGA	Study group (n = 21) formula + heat-killed <i>B. breve</i> , <i>S. thermophilus</i> and bacterial metabolites (non-digestible oligosaccharides); control group (n = 31) formula	151 weekly feces	Culture method, PCR-TTGE bacterial colonization.	Bacterial colonization, particularly <i>Bifidobacteria</i> was not modified by the type of feeding.
Romeo/ Italy/ 2011	RCT	N = 249; < 2500g BW; < 37 weeks BGA	Group I (n = 83) <i>L. reuteri</i> 1×10^8 CFU daily; group II (n = 83) <i>L. rhamnosus</i> 6×10^9 CFU daily; group III (n = 83) no probiotics.	Gastric aspirations, pharyngeal swabs and feces at birth and after 7, 14, 21 and 28 days	Cultures method; Platelia Candida test	<i>Candida</i> stool colonization was significantly higher (p < 0.01) in the control group than study group.

First Author/Region/Year	Study Design	Study Subjects	Type of Feeding	Bio-specimen	Microbiology Analysis	Major Result
Rougé/ France/ 2009	RCT	N = 94; < 32 weeks BGA; < 1500g BW	Study group (n = 45) breastmilk + <i>L. rhamnosus</i> and <i>B. longum</i> ; placebo group (n = 49) breastmilk + maltodextrin	142 feces	Culture method; temporal TTGE; prevalence and abundance of microorganism	The incidence of colonization of <i>Bifidobacteria</i> and <i>Lactobacilli</i> were significantly higher in the study group, <i>Bifidobacteria</i> was significantly higher in the study group than in the control group (p < 0.001). Study group had lower viable counts of <i>Enterobacteriaceae</i> e (p = 0.015) and <i>Clostridium spp.</i> (p = 0.014) than the control group. Antibiotic-resistant organisms didn't differ in the two groups.
Mohan/ Germany/2006	RCT	N = 69; < 37 weeks BGA	Study group (n = 37) verum with <i>B. lactis</i> ; control group (n = 32) formula-based placebo	Weekly feces	Culture method, antibiotic resisting test, FISH	The incidence of fungal colonization was significantly lower in study group than control group (p = 0.01). The number of fungal isolates obtained from each neonate (p = 0.005) and from each colonized patient (p = 0.005) were lower in study group. There were no differences in the relative proportions of the different <i>Candida</i> strains between groups.
Manzoni/ Italy/ 2006	RCT	N = 80, < 1500g BW	Study group (n = 39) MOM or DHM + <i>L. rhamnosus</i> 6 x 10 ⁹ CFU daily; control group (n = 41): MOM or DHM	Oropharyngeal, fecal, gastric aspirate, and rectal specimens on day 1, 7, 14, 21, 28, 35, and 42 of life	Cultural method, overall incidence, intensity of enteric fungal colonization, the ratio of non- <i>albicans</i> vs. <i>albicans</i> <i>Candida</i> species	<i>Bifidobacterium</i> was predominant at 2 weeks after birth in group A, 4 weeks in group B, but not dominant in group C until 7 weeks. Group A had significant earlier detection of <i>Bifidobacterium</i> and lower abundance of <i>Enterobacteriaceae</i> e at 2 weeks.
Li / Japan/ 2004	RCT	N = 30; 27.8–37.6 weeks BGA	Group A (n = 10): <i>B. breve</i> twice a day, starting several hours after birth; group B (n = 10): <i>B. breve</i> twice a day, starting 24 h after birth; control group (n = 10) no supplement.	Daily feces during the first 2 weeks after birth and then weekly to 7 weeks after birth.	Culture method	

Note. RCT = randomized controlled trial; MOM = mother's own milk; BGA = birth gestational age; BW = birth weight; HMO = human milk oligosaccharides; *B. breve* = *Bifidobacterium Breve*; *L. salivarius* = *Lactobacillus salivarius*; *B. infantis* = *Bifidobacterium infantis*; *B. lactis* = *Bifidobacterium lactis*; *L. rhamnosus* s= *Lactobacillus rhamnosus*; *S. thermophilus* = *Streptococcus thermophilus*; *L. reuteri* = *Lactobacillus reuteri*; PFGE = Pulsed-field gel electrophoresis; PCR = polymerase chain reaction; qPCR = quantitative polymerase chain reaction; TTGE = temporal temperature gel electrophoresis; TRFLP = terminal restriction fragment length poly-morphism; FISH= fluorescent in situ hybridization.