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Safety of Probiotics for Preterm Infants – A Review

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Abstract

Purpose: In absence of breast milk or in case of insufficient milk production cultured milk products developed for infants may be a practical alternative. Probiotics has now emerged as one of the most effective functional food owing to therapeutic and nutritional features. Probiotic foods have been found effective in modulating gastrointestinal flora to prevent from proliferation of diverse disease and can be used safely for infants.

Design/methodology/approach: Attempt has been made to collect literature related to probiotic application for infants. Both review and research papers related to application and safety of probiotic foods for normal and preterm infants published in diverse Journals under Pub Med and Science Direct have been considered.

Findings: Numerous reports on the efficacy of probiotic foods for healthy infants and adults have been reported. Health benefit claims of probiotic foods are still not conclusive and further studies are required to confirm its safety especially for preterm infants.

Originality/value: Probiotic foods have been found effective in modulating gastrointestinal flora to prevent from proliferation of diverse disease. Further clinical studies are emerging prior to its application for preterm infants.

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Introduction

Breast milk is considered as the most ideal food for infants owing to its numerous intrinsic therapeutic and nutritional attributes (Sarkar, 2004 a,b) besides possessing diverse range of bioactive components such as anti-microbial and anti-inflammatory factors, enzymes, hormones and growth factors (Hamosh, 2001, Field, 2005, Kainonen., *et al*. 2013, Lonnerdal, 2014). Human milk secreted by mothers delivering premature baby contains more protein and higher levels of many bioactive molecules compared to milk from women delivering full term baby and is recommended as the primary enteral diet of premature infants (Underwood, 2013).

Composition of breast milk varies considerably within feeds, across the period of lactation as well as between women (Prentice, 1996, BMA Board of Science, 2009) and exclusively breastfed infants who do not receive supplemental vitamin D are at greater risk of developing vitamin D deficiency and/or rickets (Daaboul., *et al*. 1997, Kreiter., *et al*. 2000). Dewey (2013) recommended introduction of complementary foods with a very high nutrient density particularly for iron and zinc at ages 6-12 months. However, exclusive breastfeeding is

recommended for first 6 months (Kramer and Kakuma, 2001). PAHO/WHO (2002) suggested introduction of nutritionally adequate and safe complementary food from the age of 6 months with continued breastfeeding up to or beyond 2 years of age.

WHO recommends feeding of infants with donor breast milk from another mother in case own mother fails to provide breast milk (Coutsoudis., *et al*. 2011, Kennaugh and Lockhart-Borman, 2011). Donor breast milk donated by another mother are processed at milk bank (Katke and Saraogi, 2014) but its microbiological quality is often found poor due to its contamination with Gram (-) ve bacteria and Staphylococcus through the hands of employees involved in expressing milk (de Salles and Goulart, 1997) or breast milk pumps (Boo., *et al*. 2001).Further at breast milk banks, protective effect of human milk is reported to get eliminated due to freezing and subsequent storage at 25°C for 6 months (Friend., *et al*. 1983), pasteurization (Chou., *et al*. 2001), boiling (Lawrence, 1999) or refrigerated storage (Kliegman., *et al*. 1979). Chantry., *et al*. (2011) recommended flash-heat treatment of donor milk due to retention of the bacteriostatic activity of human milk. A decline in bioactivity of breast milk resulting from degradation of protein due to heat-treatment and freeze-thawing have been encountered (Olivia Ballard and Morrow, 2013), hence human milk should be fed preferably in fresh condition in fresh condition to infants (Chou., *et al.* 2001).

Under this situation milk from various mammals such as cow, buffalo and goat may be a practical and attractive substitute. Reviewed literature indicated that human milk is virtually impossible to mimic completely, and therefore, goat's milk is often preferred over cow's milk or buffalo's milk due to its better digestibility, higher biological value and less allergenic (Sarkar, 2014). Exclusive feeding of goat milk to infants may pose risk of megaloblastic anaemia (Taitz and Wardley, 1985) therefore no mammalian milk can ideally supplement breast milk and has to be modified to come closer to human milk. Humanization of mammalian milk is necessary prior to infant feeding due to its varied composition but these products may be nutritionally adequate but could not confer any protection to the infants from infective agents. Heterogeneity in the microbiota of pre-term infants and term infants may be ascribed to extensive application of broad-spectrum antibiotic, different nursing and hygiene practice and relatively aseptic neonatal intensive care environment and there may be delayed colonization of bifidobacteria and predominance of facultative anaerobic bacteria (Gewold., *et al*. 1999).

Therapeutic properties of probiotic cultures led to the suggestion for their supplementation in infant milk with the objective of enhancing the therapeutic features. A number of cultured milk products intended for infant feeding have already been developed and found suitable for feeding both normal and sick infants (Sarkar, 2003) and health promising results due to probiotic supplementation in low birth weight infants are reported (Alfaleh and Bassler, 2008, Lin., *et al.* 2008). Meance., *et al*. (2003) annunciated the importance of probiotic dairy products towards modulating the gastrointestinal function thereby reducing the delayed gut transit which beneficially affecting the host. Probiotic supplementation for full-term infants is recommended (Wang., *et al*. 2014) but its efficacy for preterm infants to be determined prior to its recommendation. In this review paper an attempt has been taken to highlight the features of probiotics and to justify their supplementation in infant food formulations.

Health promoting properties of probiotics

The word "probiotics" have been derived from Greek, which means "for life". The Food and Agriculture Organization of the United Nation's World Health Organization defines probiotics as live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO, 2001). Probiotics confer protection against pathogens owing to their capability to compete with pathogens or their displacement by adhering to intestinal epithelial cells (Fujiwara., *et al*. 2001) and may be beneficial in the prevention or alleviation of allergic diseases in infants (Rautava and Isolauri, 2002).

Postulated health benefits of probiotics are alleviation of symptoms of lactose malabsorption, increase in natural resistance to infectious diseases of the intestinal tract, suppression of cancer, reduction in serum cholesterol concentration, stimulation of GI immunity (Collins and Gibson, 1999), improve intestinal tract, enhanced immune system, synthesis and enhanced the bioavailability of nutrients, prevent allergy in susceptible individuals (Kopp-Hoolihan, 2001).

Diverse health benefits extended by probiotics towards undesirable flora may be resultant of different mechanisms of probiotic effect. Various mechanisms of probiotic actions are decreasing the luminal pH by the production of short chain fatty acids, rendering vital nutrients unavailable to pathogens, altering the redox potential of the environment (Kailasapathy and Chin, 2000), restoration of increased intestinal permeability (Dinkci., *et al*. 2006), enhancing host defines mechanisms, modulating host immune response (Saavedra, 2007), production of antimicrobial substances up regulating immune response to possible pathogens, down regulating inflammatory response, assist in early programming of the immune system, improve gut mucosal barrier function, promoting recovery of commensal microbiota, modulating host gene expression, delivering functional proteins or enzymes, decreasing pathogen adhesion (Sanders, 2009), secreting antimicrobial peptides, inhibiting bacterial invasion, increasing mucus production, effects on dendritic cells, monocytes/macrophage, lymphocytes, NK cells, T cells, T cell redistribution (Ng., *et al*. 2009).

Modulation of gut flora by probiotic

Gut colonization is a continuous phenomenon which starts at birth and is seeded with microorganisms due to swallowing of colonized amniotic fluid (DiGiulio., *et al.* 2008) and from various sources such as environment, maternal vagina and faeces (Rotimi and Duerden, 1981, Dominguez-Bello., *et al*. 2010) and constituted of facultative anaerobes such as enterobacteria, *Streptococcus* and *Staphylococcus*. *Bifidobacterium* and *Lactobacillus* species appear after the first week and the former remained as the predominant component of the gut microbiota in infancy (Balamurugan., *et al*. 2010) and act as a physical and immunologic barrier between the host and the environment and maintain a disease-free state in the gastrointestinal tract (Salminen and Isolauri, 2006).

Preterm infants are particularly susceptible to abnormal colonization. Premature newborns in the intensive care acquire colonizing bacteria from the intensive care microenvironment rather than their mother due to less exposure to the maternal microbiota, multiple courses of antibiotics and delays in feeding and harbour a bacterial flora composed of predominant aerobes such as *Staphylococcus aureus*, *Klebsiella* and enterococci, whereas the predominant anaerobes include Clostridia (Gewold., *et al*. 1999). Diversity in gut flora of preterm infants and term infants and delayed bifidobacteria colonization coupled with higher prevalence of *Clostridium difficile* in preterm infants may be attributable to the use of parenteral nutrition and antibiotic therapy for extended periods.

Preterm infants have immature physiological systems due to an underdeveloped gastrointestinal resulting in translocation of potentially pathogenic bacteria from the intestinal lumen causing systemic infections. A balance between beneficial and pathogenic microorganisms exists in the human gut and when the homeostasis of microorganisms get disrupted due to antibiotic treatment or in some disease situations there is an increase in pathogenic bacteria resulting in disturbance in microbial equilibrium. Various factors such as the immature intestinal function, frequent use of broad-spectrum antibiotics, delay in initiating enteral feeding, infection control procedures and sterilization of milk limit the exposure of preterm infants to normal commensal microorganisms and are therefore prone to systemic infections due to increased intestinal permeability to potentially pathogens.

Probiotics exhibits therapeutic effects by positively influencing normal microbe-microbe and host–microbe interactions, augmenting the protection against infections by commensal flora through competitive interactions, direct antagonism of pathogens, and/or production of antimicrobial factors or by altering the intestinal lumen pH by producing potentially microbicidal short-chain volatile fatty acids, which may inhibit the proliferation of pathogenic microorganisms. Rijkers., *et al*. (2010) categorized the diverse health benefits extended by probiotics into three groups.

- Probiotic microorganisms act directly within the GI tract through direct interaction with the intestinal microbiota or by enzymatic activities.
- Probiotic microorganisms interact directly with the intestinal mucus layer and epithelium thereby influencing the intestinal barrier function and the mucosal immune system.
- Probiotic microorganisms can have effects outside the GI tract such as on the systemic immune system and other organs.

Probiotics for normal infants

A recent double-blind, randomized controlled trial revealed that probiotic supplementation was efficacious in full-term infants with critical illness and induced a significantly reduced rate of nosocomial pneumonia (18% vs. 36%) and multiple organ dysfunction syndrome (6% vs. 16%) compared with the placebo group (Wang., *et al.* 2014). Reviews on clinical report on the use of probiotics indicated modestly effectiveness in treating acute viral gastroenteritis and preventing antibiotic-associated diarrhea but preliminary encouraging results for the treatment childhood *Helicobacter pylori* gastritis, irritable bowel syndrome, chronic ulcerative colitis and infantile colic in healthy children and require further confirmation (Thomas., *et al*. 2010). Due to failure of probiotics administration for reducing the incidence of diarrhoea (Mugambi., *et al*. 2012 b) in full term infants and insufficient evidence application of probiotics in prevention and treatment of diverse diseases is not recommended and requires more studies as specific probiotics exhibited specific health benefits (Vandenplas., *et al*. 2011). Based upon limited evidence use of probiotics is suggested for an improvement in child growth in developing countries and in under-nourished children, however more research is needed for further exploration (Onubi., *et al*. 2015).

Probiotics for premature infants

Probiotics prevented gut colonization by Candida and conferred protection against sepis and abnormal neurological outcomes in preterms and greater efficacy of *L. reuteri* than *L. rhamnosus* (Romeo., *et al.* 2011) may be attributed to a lower colonization of *L. rhamnosus* in preterm infants with a birth weight < 1500g than in those with a birth weight between 1500 and 1999g (Pappas, 2004). Probiotic supplementation comprising *Bifidobacterium infantis, Lactobacillus*, and *B. lactis* to preterm modulated the intestinal microflora and minimize enteral fungal colonization, reduced invasive fungal sepsis, earlier establishment of full enteral feeds, and reduced duration of hospital (Roy., *et al.* 2014). Reviewed literature provided evidence-based guidelines which indicated that Lactobacillus GG alone may not be effective but a probiotic combination comprising of Lactobacillus and at least one Bifidobacterium species at a daily dose level of 3×10^9 organisms must be initiated within first 7 days of life and to be continued for at least until 35 weeks corrected age or discharge in preterm neonates (Lin., *et al*. 2008). Recent reports indicated that administration probiotic such as *B. infantis, S. thermophilus* and *B. lactis* (Jacobs., *et al.* 2013) or *Lactobacillus reuteri* (Shadkam., *et al*. 2015) may be safe and is diminishing the incidence of NEC in very low birth weight premature infants.

Vandenplas., *et al*. (2015) considered *Lactobacillus rhamnosus* GG and *Saccharomyces boulardiias* safe due to no report of its adverse effects but evidence of clinical benefit is accumulating. However, development of sepsis in newborns and children due to supplementation of *Lactobacillus rhamnosus* GG conclude that such supplementation may not be safe for high-risk patients on rare occasions (Carlo., *et al.* 2016) and did not recommend for incorporating routine probiotic prophylaxis in clinical practice (Shlomai., *et al.* 2014).

Probiotic supplementation induced colonization of intestinal flora with *Bifidobacterium lactis* Bb12 in preterm infants and resulted in beneficial effects on survival, infection rate, and incidence of NEC (Thomas., *et al*. 2010) and a recent double-blind, randomized, controlled clinical trial concluded that oral probiotic supplementation with *B. breve* and *L. casei* reduced the occurrence of NEC (Braga., *et al*. 2011). Based upon Randomized Controlled Trials (RCT) administration of probiotic in preterm infants with a birth weight > 1000g could be recommended due to significantly reduction in incidence of NEC (Alfaleh and Bassler, 2008) and no systemic infections or serious adverse events was reported. Recently systematic reviews of randomized, controlled trials indicated lower mortality and NEC in very low birth weight neonates (Deshpande., *et al*. 2010) and facilitated enteral feeds in preterm infants (Shlomai., *et al*. 2014).

Safety of probiotic therapy for preterm infants

Though health benefits of probiotics have been established and recommended for application as a pharmaceutical agent but certain gaps still remains for their extensive exploitation. Certain contradictory reports on the efficacy of probiotics for pre-term infants have been reported.

- Prophylactic effect of probiotics should not be generalized for others without separate confirmatory studies (Boyle., *et al*. 2006) and long term studies regarding its safety must be evaluated prior to its routine practice especially for preterm infant (Millar., *et al*. 2012).

- Clinical studies indicated that probiotics may not be equally effective for all disease or disorder (Reid., *et al*. 2010), all patient groups especially those born prematurely or with immune deficiency (Boyle., *et al*. 2006) and is influenced by specific strains employed, dosage and duration of administration of probiotics (Gawronska., *et al.* 2007).
- Administration of probiotics failed to significantly reduce the risk of sepsis and NEC in preterm infants (Stratiki., *et al*. 2007) and more studies are needed to determine the efficacy of probiotics for NEC (Thomas., *et al.* 2010).
- Based upon data meta-analysis probiotic cannot be recommended for breastfed infants with colic or for routine use for preventing infantile colic (Sung., *et al.* 2014).
- No colonization by probiotic strains was detected in infants who weighed ≤1000g, presumably because of more frequent suspensions of enteral feeding, more courses of antibiotic treatment, or both (Rouge., *et al*. 2009).
- Supplementation with *Bifidobacterium longum* BB536 and *Lactobacillus rhamnosus* GG may not improve the gastrointestinal tolerance to enteral feeding in very-low-birth weight infants but may improve gastrointestinal tolerance in infants weighing > 1000g (Rouge., *et al*. 2009).
- Administration of *Lactobacillus acidophilus* at a level of 108 cfu induced colonization of gastrointestinal tract with Lactobacillus in 37% preterm infants and improved feeding tolerance (Lee., *et al.* 2007).
- Enteral feeding of premature infants with *Lactobacillus* sp. strain GG showed survival of the organism through the GIT but could not confer any detectable benefits (Millar., *et al.* 1993).
- For premature infants optimal strains and dose regimens are yet to be examined closely (Chauhan., *et al*. 2008).
- Due to insufficient evidence for improved growth and clinical outcomes supplementation of probiotics for exclusively formula fed preterm infants is not suggested (Mugambi., *et al.* 2012 a).
- Routine use of probiotics in the preterm infant could not be recommended due to many uncertainties such as the mechanisms of action of probiotics, health effects of employed probiotics, determination of reasons for the efficacy of probiotics, forms of microbial adaptations and ecological consequences (Millar., *et al.* 2011).
- Routine probiotic supplementation is justified for preterm infants except for ELBW (Deshpande., *et al.* 2009) and further investigations are required to assess the potential benefit and safety of probiotic supplementation for extremely low birth weight infants (Thomas., *et al.* 2010).

Recommendations

- Probiotic supplementation in preterm neonates exhibited good safety profile and did not show any side effects however more randomized controlled trials to evaluate safety profile of probiotic supplementation for the prevention of NEC in extremely low birth weight infants and as an additive treatment to prevent invasive fungal infections in preterm neonates are emerging.
- Optimal probiotic strain, duration of administration and host selectivity remain unclear due to heterogeneity of trial design and therefore repeated studies using a single design protocol to demonstrate reproducibility, safety and efficacy are suggested.
- Suggestion for an international standard for probiotic trials on human health may facilitate in the comparison of results from different probiotic products to arrive upon a conclusive result. International Scientific Association for Probiotics and Prebiotics (ISAPP) had given four recommendations to achieve consistent health benefits due to probiotic supplementation.
- Clearly define the end goal
- Design the study
- Base the selection of the intervention on scientific investigations
- Carefully select the study cohort

Conclusion

Clinical studies indicated that probiotics may not be equally effective for all disease or disorder, all patient groups especially those born prematurely or with immune deficiency and is influenced by specific strains employed, dosage and duration of administration of probiotics. Routine use of probiotics in the preterm infant is not be recommended due to many uncertainties such as the mechanisms

of action of probiotics, health effects of employed probiotics, forms of microbial adaptations and ecological consequences. A large randomized controlled trial is required to investigate the potential benefits and safety profile of probiotics supplementation in extremely low birth weight infants.

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