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The Prophylactic Role of probiotics for Preterm Neonates

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ABSTRACT

Background: Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. Probiotics have been used for prevention and treatment of various medical conditions in children and adults. Studies on probiotics in premature infants have focused on normalizing intestinal flora, improvement in feeding intolerance, prevention of necrotizing enterocolitis which is the leading causes of death in the neonatal intensive care unit.

Objective of the Study: was to provide an overview of the controversies regarding probiotic use in preterm infants and to shed light on the practical considerations for implementation of probiotic supplementation.

Methods: A Systematic search in the scientific database (Medline, Scopus, EMBASE , and Google Scholer) from 1990 to 2016 was conducted for all relevant retrospective studies including; retrospective , prospective and randomized controlled trials and cohort studies were analyzed and included based on the preset inclusion and exclusion criteria.

Results: The search results yielded 16 studies, 12 of which were RCTs with 2340 premature neonates and 4 meta-analyses with 10227 neonates which showed a significantly decreased incidence of Necrotizing Enterocolitis (NEC) (risk ratio, RR = 0.35, 95% confidence interval, 95% CI, 0.23-0.54; p = 0.0006) and mortality (RR = 0.46, 95% CI, 0.32-0.67; p < 0.0001). Sepsis did not differ significantly between the two groups (RR = 0.93, 95% CI, 0.76-1.15; p = 0.05).

Conclusion: there is a strong body of evidence supporting that Probiotic supplementation reduces the risk of NEC and mortality in preterm infants yet there is no sufficient evidence to support the optimal strain, dose and timing need further investigation.

Keywords: probiotics, Neonates, Lactobacillus reuteri, necrotizing enterocolitis, premature infant

INTRODUCTION

The early bacterial pattern in the first weeks of life appears to be a crucial step in the establishment of the various functions of the gut microbiota. In fact, recognition of self– and non–self–antigens begins early in life, perhaps even in utero[1].

Maturation of the intestinal immune system is thought to be significantly affected by the sequential bacterial establishment[2]. Indeed, at birth, the lymphoid system is not yet mature even though it is developed and the fetus is in a Th2 immunological context, and Th1 responses are repressed in order to avoid its rejection[3] .Therefore, after birth, the newborn must quickly restore the Th1/Th2 balance. The existence of a rich microbial environment is thought to be important in this process, the first bacteria to colonize the infant's gut being the first stimuli for post-natal maturation of the T-helper balance.

The immature Th2- dominant neonatal response undergoes environment-driven maturation via microbial contact during the early postnatal period resulting in a gradual inhibition of the Th2 response and an increase of the Th1 response and prevention of allergic diseases which are Th2 linked, a basis of the so-called "hygiene hypothesis" [2].

Late-onset diseases could be therefore associated with an impairment of this step, all the more as early impairment in bacterial establishment can have long term effects in terms of bacterial pattern[4].

Factors known to modify establishment of the gut microbiota, e.g. birth through caesarian section[5], prematurity[6], and exposure to antibiotics during pregnancy[7] have been associated with a higher risk of atopic disease. This hygiene hypothesis implicating a

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relationship between allergic diseases and gut microbiota is supported by several clinical studies which reported differences in the composition of the fecal microbiota between infants who live in countries with high or low prevalence of allergy, as well between infants with or without allergic diseases.
Hence, although a causal relationship has not been categorically established, there is emerging evidence that the initial gut bacterial colonization during the first weeks of life is of great importance for infant health. Perinatal determinants altering the colonization pattern could therefore lead to a higher risk of later diseases[8]. For instance, infants born through cesarean section and therefore colonized by an altered bacterial pattern as compared with vaginally delivered ones have been reported to be at higher risk of either allergic diseases[6], or celiac disease[9], or obesity[10], or type 1 diabetes[11]. A prolonged breast-feeding over one year has been linked to a lower risk of overweight or obesity[12]. Likewise, changes in the establishment of gut microbiota observed in modern Western infants result in reduced bacterial exposure[13]. Thus, these infants lack of adequate bacterial stimuli, leading to a deviated maturation of their immune system likely responsible for a higher risk of allergic disease development or inflammatory bowel diseases[2].

Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host [14]. The term probiotics was initially used in the 1960s and comes from the Greek word meaning “for life.” Probiotics are commonly available as supplements (capsules, tablets, packets, or powders) and fermented dairy products such as yogurt. An ideal probiotic agent must be healthy, resist degradation by gastric acids and bile salts, adhere to intestinal epithelial cells, be considered nonpathogenic and non-invasive, modulate immune responses, be sensitive to usual antibiotics without the development of resistance, originate from microflora, and resist technological processing[15].

The common microorganisms used as probiotics include (a)Bacteria; (i)Lactobacillus species: L. rhamnosus GG, L. acidophilus, L. caseii, L. plantarum, L. lactis, L. reuteri, and so forth;(ii)Bifidobacterium species: B. bifidum, B. breve, B. infantis, B. lactis, and B. longum;(iii)Streptococcus thermophiles;(b)Yeast: Saccharomyces boulardii[16].

The potential benefits of the use of probiotics in pediatrics have been reviewed[17]. It mainly includes treatment acute viral gastroenteritis[18], prevention of **antibiotic associated** diarrhea[19], reduction of the inflammatory response in inflammatory bowel disease (IBD) patients. Limited effects have been observed in colicky infants[20]. However, a study reported a clear improvement of the symptoms of colic within one week of Lactobacillus reuteri administration as compared with simethicone treated infants[21] linked to an antimicrobial effect against six species of gas-forming coliforms isolated from the colicky infants[22].

Given the likely link between the early bacterial pattern and later health status reported, a very early administration of probiotics when the gut microbiota is not fully established is of great interest and we have focused this review on this approach. Many attempts of early probiotic supplementation have been made for a long time, and numerous studies related to the use of infant formula supplemented with probiotics strains have been published as well [23]. This early use is reported to have some beneficial effects in terms of prevention of late development of some diseases. Administration is often given soon after birth, and the duration is variable according to the study, but often prolonged over several weeks or months. Lastly, dosages varied, ranging from 106 to ~109 CFU/mL or/g. The most frequently studied probiotic strains were Bifidobacterium animalis subsp lactis, B longum, Lactobacillus rhamnosus, L reuteri, L johnsonii and Streptococcus thermophilus, used alone or in combination.

The immature immune system of premature neonates cannot control the outgrowth of pathogenic bacteria. According to the benefits of probiotics, feeding premature infants with these bacteria may populate their intestines with normal flora and prevent an overgrowth of pathogenic flora that contribute to the development of NEC[24].
Necrotizing enterocolitis (NEC) remains an important cause of morbidity and mortality among very preterm infants. Furthermore, Despite the advances in neonatal intensive care over the period 1986-2006[25], the incidence of

necrotising enterocolitis (NEC) in preterm neonates has not changed significantly. The mortality (approximately 20 to 25%) and morbidity related to definite (greater than stage II) NEC, including prolonged hospitalisation[26], survival with short-bowel syndrome and long-term neurodevelopmental impairment (NDI) continues to be high, especially in preterm or extremely low birth weight (ELBW) (birth weight < 1000 g, gestation < 28 weeks) neonates needing surgery for this illness[27].

Furthermore, Mortality reaches nearly 100% in children with extensive and full-thickness necrosis of the gut[28]. Despite many investigations, its pathogenesis remains unclear.The hypothesis that intestinal microbes are necessary for the development of NEC is supported by several lines of evidence[29].

No specific bacteria or bacterial pattern has been causally associated with the development of NEC although bacterial colonization is recognized as an important factor[30].

The present systematic review aimed at giving the rational of the use of probiotics for promotion of health and prevention of disease through their use early in life; for Preterm Neonates.

MATERIALS AND METHODS

Literature search

The present Systematic Review is reported in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Data Sources: electronic databases were searched: Scopus, EMBASE , and Google Scholer) , PubMed/MEDLINE, Scopus, The Cochrane Library, and Web of Science. Econlit from 1990 to 2016.

Search terms included "Neonates" "AND probiotics "AND "NEC".

STUDY SELECTION

Study Selection:

Search results were screened by scanning abstracts for the following:

Inclusion Criteria

1. Nonrandomized studies comparing prophylactic probiotics to a standard regime for preterm infants.
2. Gestational age <37 weeks or birth weight <2,500 g
3. Studies had to include at least 20 participants.
4. All probiotic regimes were included, as well as combinations (e.g. Lactobacillus acidophilus and Bifidobacterium lactis),
5. the intervention had to be administered for at least 7 days

Exclusion Criteria

1. Neonates with significant birth defects (e.g. severe heart disease, myelomeningocele).
2. Age >37 weeks or birth weight >2,500 g.

Data Extraction and Study Quality Assessment

The quality of included trials was assessed by R.O. and J.B. using the Newcastle-Ottawa Scale (NOS) [31], which was modified to fit our study design: 0-3 stars indicate poor study quality, 4-6 stars indicate acceptable study quality, and 7-9 stars indicate good study quality. In the event of disagreements, consensus was reached by discussion.

RESULTS

The initial search was broad, accepting any article related The Prophylactic Role of probiotics for Preterm Neonates to ensure a comprehensive view of available work. Searches identified 423 publications in addition to another 13 publications that were found through manual research. After removal of duplicates, abstracts and titles 211 publications were assessed as identified from title and abstract, 132 papers were again excluded after another scrutinizing round,10 papers full text could not be retrieved and another 26 papers with the same cohort and 67 because they did not have the same endpoint (didn't conclude or touch base on probiotics prophylactic effect for neonates or preterm infants).

Finally 12 eligible articles met the inclusion and exclusion criteria and detailed as the focus for the present study.

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines in reporting the results [17].

Figure 1

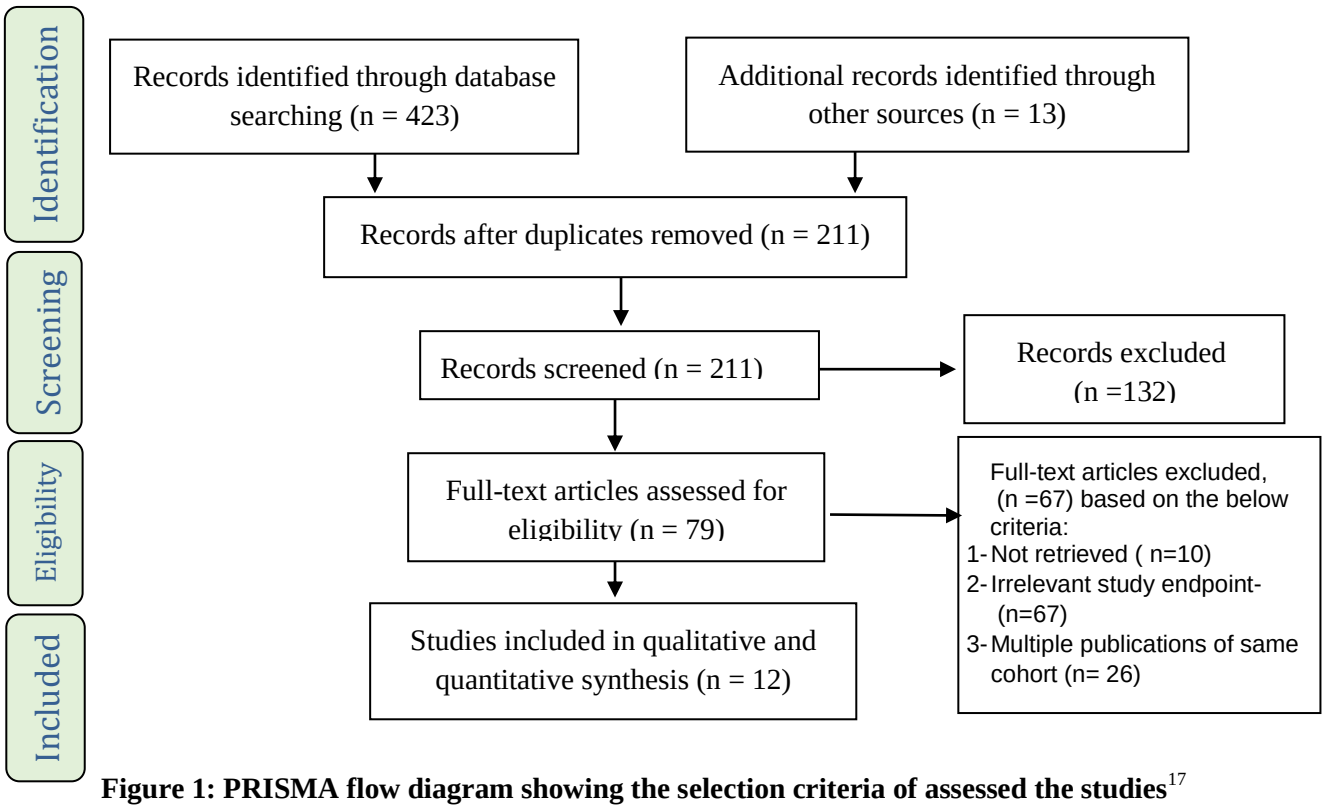

Figure 1: PRISMA flow diagram showing the selection criteria of assessed the studies[17]

Evidence by the included studies (clinical trials of Probiotics for Prevention of Sepsis and NEC in Preterm Infants):

Dani *et al.* [32] reported a double-blind RCT in 585 preterm VLBW infants to determine the effectiveness of *Lactobacillus GG* on urinary tract infection (UTI), bacterial sepsis, and NEC at 12 NICUs in Italy. No significant differences were observed between the groups: UTI (3.4% versus 5.2%), sepsis (4.7% versus 4.1%), and NEC (1.4% versus 2.8%). Awad *et al.* [33] examined the role of live and killed *Lactobacillus acidophilus* in reducing the incidence of nosocomial sepsis and NEC in 150 neonates (including 89 preterm infants). Infants who received either live or killed *Lactobacillus acidophilus* were less likely to develop nosocomial sepsis (45% versus 53.3% versus 63.3%), but it did not reach statistical significance.

In an RCT by Mihatsch *et al.*[34], 183 VLBW infants <30 weeks of gestation were randomly assigned to have their milk feedings accompanied with *Bifidobacterium lactis* or placebo for the first 6 weeks of life. Primary outcome was the "incidence density" of nosocomial infections defined as periods of elevated C-reactive protein (>10 mg/L) from day 7 after commencement of milk feedings until the 42nd day of life (number of nosocomial infections/total number of patient days). There was no significant difference between the two groups with regard to the incidence density of nosocomial infections and the actual number of nosocomial sepsis. Reassuringly, none of the blood cultures grew *Bifidobacterium lactis.*

Romeo *et al.* [35] evaluated the role of probiotics for prevention of enteric Candida colonization and late onset sepsis in 249 preterm infants. The infants were randomized into three groups; one group supplemented with *Lactobacillus reuteri (LR)*, the second group supplemented with *Lactobacillus rhamnosus (LGG)*, and third group with no supplementation (control). The mean gestational age was 33 weeks. Candida stool colonization was significantly higher in control groups as compared with the probiotics groups. Only one infant in the LR group developed nosocomial sepsis; two infants in the LGG group developed nosocomial sepsis and nine infants in the control group developed nosocomial sepsis.

Table 1 shows the details of randomized control trials (RCT) describing the impact of Probiotics on the neonatal outcome [32–43].The primary outcome is NEC in majority of the trials and nosocomial sepsis is often a secondary outcome. We summarized the clinical trials on probiotics with nosocomial sepsis as one of the primary outcomes.

Table 1: Studies included of clinical trials of probiotics for prevention of NEC and sepsis in neonates.

Study characteristics			GA (wk)	Probiotic used	Dose and duration	Primary outcome	Comments
Publication	**Year**	**Country**	**BW (g)**				
Dani *et al.* [32]	2002	Italy	<33 <1500 *N* = 585	*LGG*	6×10^9 CFU once daily from first feeds till discharge	Urinary tract infection, bacterial sepsis, NEC	No difference in all three outcomes
Awad *et al.* [33]	2010	Egypt	All neonate *N* = 150	*LA* (live and killed)	6×10^9 CFU twice daily from day 1 till discharged	Sepsis and NEC	↓ sepsis rate in probiotic groups
Mihatsch *et al.* [34]	2010	Germany	<30 and <1500 *N* = 183	*BL*	12×10^9 CFU/Kg/day for 6 weeks	Incidence density of nosocomial infection	No difference in sepsis
Costalos *et al.* [35]	2003	Greece	28–32 *N* = 87	*SB*	10^9/kg twice daily from first feed for 30 days	Gut function and stool colonization	No difference in sepsis
Lin *et al.* [36]	2005	Taiwan	<1500 *N* = 367	*LA, BI*	LA: 1004356 BI: 1015697 twice daily from day 7 until discharge	NEC	↓ NEC and sepsis rate in probiotic group (12.2% versus 19.3%)
Manzoni *et al.* [37]	2006	Italy	<1500 *N* = 80	*LBC*	6×10^9 CFU once daily from third day of life to 6 wks or discharge from NICU	Gut colonization by Candida	No difference in sepsis
Stratiki *et al.* [38]	2007	Greece	27–37 *N* = 78	*BL*	Preterm formula 2×10^7 CFU/g started within 48 h.	Intestinal permeability	No difference in sepsis
Samanta *et al.* [39]	2009	India	<32 <1500 *N* = 186	*BI, BB, BL, LA*	2.5×10^9 CFU/day till discharge	NEC, feed tolerance	↓ Sepsis in probiotic group (14.3% versus 29.5%)
Rougé *et al.* [40]	2009	France	<32 <1500 *N* = 94	*BL, LGG*	1×10^8 CFU per day until discharge	Enteral feed intake at day 14	No difference in sepsis (33.3% versus 26.5%)
Romeo *et al.* [41]	2011	Italy	<37	*LR*	LR: 1×10^8 CFU daily	Gut fungal colonization and late onset sepsis	Probiotics effective in prevention of gut colonization by Candida.
			<2500	*LGG*	LGG: 6×10^9 CFU daily from first 72 hrs to 6 wks or until discharge		No difference in sepsis
			N = 249				
Sari *et al.* [42]	2011	Turkey	<33 <1500 *N* = 221	*LS*	3.5×10^9 till discharged	NEC, and mortality	No difference in sepsis (26.4% versus 23.4%)
Fernández-Carrocera *et al.* [43]	2013	Mexico	<1500 *N* = 150	*LA, LGG, LC, LP, BI, ST*	Multispecies probiotics 1 g/day	NEC	No difference in NEC and sepsis rate (56% versus 58.7%)

BB: Bifidobacterium bifidus; BL: Bifidobacteruim lactis; LB: Bifidobacterium breve; LGG: Lactobacillus rhamnosus GG; LS: Lactobacillus sporogenes; SB: Saccharomyces boulardii; BBr: Bifidobacteria breve; BLo: Bifidobacterium longum; LC: Lactobacillus casei; NEC: necrotizing enterocolitis; *ST: Streptococcus thermophillus; BI: Bifidobacterium infantis;* CFU: colony forming units; *LP: Lactobacillus plantarum; LR: Lactobacillus reuteri*

Four meta-analyses and two systematic reviews on probiotics in preterm infants have been published [42–45]. The details of the meta-analyses are shown in Table 2.

Table2: Meta-analyses of probiotics in neonates.

Authors	Publication year	Number of trials	Inclusion criteria	Number of infants	Sepsis (RR; 95% CI)	NEC (RR; 95% CI)	Mortality (RR; 95% CI)
Wang *et al.* [42]	2012	20	<34 wks <1500 g	3816	0.90; 0.71–1.15	0.33; 0.24–0.46	0.56; 0.43–0.73
Alfaleh *et al.* [43]	2011	16	<37 wks <2500 g	2842	0.90, 0.76–1.07	0.35, 0.24–0.52	0.40, 0.27–0.60
Deshpande *et al.* [44]	2010	11	<34 wks <1500 g	2176	0.98; 0.81–1.18	0.35; 0.23–0.55	0.42; 0.29–0.62
Deshpande *et al.* [45]	2007	7	<33 wks <1500 g	1393	0.94; 0.74–1.20	0.36; 0.20–0.65	0.47; 0.30–0.73

DISCUSSION

The numerous reviews and meta analyses conducted strongly suggest that the use of probiotics in preterm infants could prevent tens of thousands of deaths annually. Hence, some authors recommend that it is time to change practice and to adopt the use of probiotics as a standard care in preterm infants[46]. However, controversies have emerged because there are yet too many unknowns about probiotics use . One aspect concerns the safety although no negative effects have been reported even in long term follow-up[47]. However, data on this latter aspect are very scarce. Infrequent, systemic translocation of probiotics has been reported raising some concerns about this side effect in the high-risk groups of low and very low birth weight infants who are characterized by high intestinal permeability, making this potential powerful tool a double-edge weapon. Increased incidence of NEC following probiotic administration has been observed in a preterm piglet model, may be related to the specific strain, dose, and the very immature gut immune system[48]. A study in a pediatric unit even reported a trend toward an increase in nosocomial throughout a probiotic supplementation although a routinary supplementation of VLBW infants with a probiotics strains over a 6- year period was safe [49].

LIMITATIONS OF THE STUDY

The available and included trials did not look at one specific product, dosing regimen, or protocol. Methods of randomization, blinding, and feeding regimens were vague or unpublished. Some authors have stated that the studies published up to 2011 were underpowered to establish any appropriate conclusions[50] .Future studies should not be focused on questioning the benefits of probiotics, rather they should further delineate the ideal probiotic, target group, and duration of therapy. Despite the lack of consensus regarding the benefit of probiotics, many NICUs are routinely giving probiotic supplements to preterm infants. From the current data, it appears that NICUs with high incidence rates of NEC are more likely to benefit from probiotic supplementation. Although multistrain products may be more effective than single-strain products, evidence is still lacking in this area, along with their efficacy in extremely low-birth-weight infants.

CONCLUSION

There is strong body of evidence from clinical trials suggesting that probiotic supplementation has a significant role in the prevention/minimization of mortality and morbidity specifically NEC in neonates. However, there is no evidence regarding the usefulness of either probiotics or prebiotics for the prevention of nosocomial sepsis in preterm infants. Thus, results from multicentre trial powered to conduct more studies for sufficient data and evidence for recommending routine probiotics for all neomates on safety and efficacy of probiotics are awaited.

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