Original Article

Role of Enteric Supplementation of Probiotics on Late-onset Sepsis by Candida species in Preterm Low Birth Weight Neonates: A Randomized, Double Blind, Placebo-controlled Trial

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Abstract

Background: The increase in invasive fungal infections (IFIs) in neonatal intensive care unit (NICU) is jeopardizing the survival of preterm neonates. Probiotics modulating the intestinal microflora of preterm neonates may minimize enteral fungal colonization. **Aims:** This study was to examine whether probiotic supplementation in neonates reduced fungal septicemia. **Materials and Methods:** This prospective, randomized, double blind trial investigating the supplementation of preterm infants with a probiotic was done from May 2012 to April 2013, with 112 subjects randomized into two groups. Primary outcome: Decreased fungal colonization in gastrointestinal tract. Others: Incidence of late onset septicemia; duration of the primary hospital admission; number of days until full enteral feeds established. **Results:** Full feed establishment was earlier in probiotics group compared to placebo group ($P = 0.016$). The duration of hospitalization was less in the probiotic group ($P = 0.002$). Stool fungal colonization, an important outcome parameter was $3.03 \pm 2.33 \times 10^5$ colony formation units (CFU) in the probiotics group compared to $3 \pm 1.5 \times 10^5$ CFU in the placebo group ($P = 0.03$). Fungal infection is less in the study group ($P = 0.001$). **Conclusion:** The key features of our study were reduced enteral fungal colonization, reduce invasive fungal sepsis, earlier establishment of full enteral feeds, and reduced duration of hospital stay in the probiotics group.

Keywords: Fungal, neonates, preterm, probiotics, sepsis

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Introduction

Candidemia in the neonatal intensive care unit (NICU) is the third most frequent causal agent of late-onset sepsis in preterm neonates affecting 1.6-9% very low birth weight (VLBW) and 15% extremely low birth weight (ELBW) neonates.[1-4] The increased frequency of invasive fungal infections (IFIs) in NICU is questioning the survival of preterm neonates and neurodevelopmental outcome.[5]

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In healthy term infants, colonizations of the aseptic intestine are acquired from the birth canal, subsequently modified by diet.[6] However, preterms in NICU acquire colonizing bacteria from the intensive care microenvironment rather than their mother.[7]

In NICU preterms, intestinal functional immaturity, broad-spectrum antibiotics, and delay in initiating enteral feeding prevent the enteric colonization with normal commensal microorganisms. Thus, they harbor aerobes like Staphylococci (coagulase negative and *Staphylococcus aureus*), Enterobacteria (Klebsiella), Enterococci, and anaerobes like Clostridia.[8] Normal commensal bacterial flora inhibits Candida growth by competing for adhesion sites and nutrients.[9] The use of H2 blockers is another risk factor for Candida infection.[10] Preterms have an abnormal pattern of gut colonization with bifidobacteria and lactobacilli which normally colonize healthy full-term infants.[7,11] This altered

intestinal flora increase their susceptibility to necrotizing enterocolitis and risk of bacterial translocation.[12,13]

Of the risk factors, fungal colonization by Candida species is common for any IFI.[4-14] Sixty percent of VLBW neonates become colonized by fungi during the 1st month of NICU life and 21% of them progress to become infected.[14]

Of all colonization sites[14,15] the gastrointestinal tract is most frequently implicated in subsequent systemic fungal dissemination.[16] Thus, reducing fungal colonization can prevent IFI. Systemic antifungal drugs have shown promising results, but antifungal prophylaxis raises concerns about selection of resistant strains.[17-19]

Probiotics, defined as live microbial supplements providing health benefits to the host may modulate the intestinal microbiota in preterms.[20] The bacteria most frequently used as probiotics are the bifidobacteria and lactobacilli. Probiotics may prevent gastrointestinal and urinary infections by: Increasing resistance of mucosal barrier to migration of bacteria and their toxins by strengthening intestinal cell junctions, increased host response to microbes, and increased mucosal immunoglobulin A response, inhibit the growth of pathogens, production of bacteriocins, and competitive exclusion of potential pathogens.[21,22] Two meta-analyses based on an aggregate of seven and nine clinical trials concluded that neonatal probiotic supplementation reduces the incidence of necrotizing enterocolitis (NEC) in preterm infants, diarrhea, colon distension, and abdominal cramps and less time required to reach full enteral feeding.[23-28] But, the safety and efficacy of probiotics in preterms especially ELBW is yet to be proven.[24]

Our hypothesis was that increased colonization with beneficial microflora like probiotics would protect the neonate host from the expansion of fungal colonies in the gastrointestinal tract.[29] In this field, some trials have been conducted, but some do not include preterms weighing less than 1,000 g and before 28 weeks' gestation. Data regarding the optimal strain (s), dose, time to start, and duration of treatment of currently available probiotics are lacking.

The objective of this study was to evaluate the hypothesis that supplementation with probiotics may reduce the colonization and expansion of fungal colonies in the gastrointestinal tract, and reduce the risk of bacterial and/or fungal late onset sepsis in the NICU.

Materials and Methods

This is a single-center, prospective, randomized, double blind, placebo-controlled trial investigating the supplementation of preterm, LBW infants with a probiotic combination comprising *Bifidobacterium infantis*, Lactobacillus, and *B. lactis*.

Institutional approval was obtained from the ethics committee of our institution and Clinical Trial Registry of India (CTRI) registration number REF/2012/12/004378. Written informed consent was obtained from the parents prior to study and confidentiality was maintained throughout the study.

In the NICU of a tertiary care hospital of eastern India from May 2012 to April 2013, a total of 341 subjects are assessed for eligibility and 112 satisfying the inclusion and exclusion criteria (mentioned below) were randomized into two groups [Figure 1]. The mean delivery per year was 9,000 with average NICU admission of 600 per year.

Inclusion criteria were admission to the NICU, a stable oral feeding within 72 h of birth and an informed parental consent; gestational age (GA) < 37 weeks; birth weight < 2,500 g; adequate renal and liver function; a postnatal age < 2 week; did not have baseline fungal colonization at enrollment (with colonization defined by isolation of fungi from a culture specimen obtained from any site during the first 3 days of life); did not receive any form of antifungal prophylaxis other than the probiotic used.

Exclusion criteria were the presence of major congenital malformation; antenatal and perinatal risk factors for sepsis, major congenital malformation; stigma of congenital infection; severe lesions diagnosed by cranial ultrasound (e.g. intraventricular hemorrhage (IVH) grade 3 and 4 and major ischemic lesions); altered liver and renal function; likely to die within 72 h of birth; and babies of mothers taking supplemental probiotics by capsule/powder.

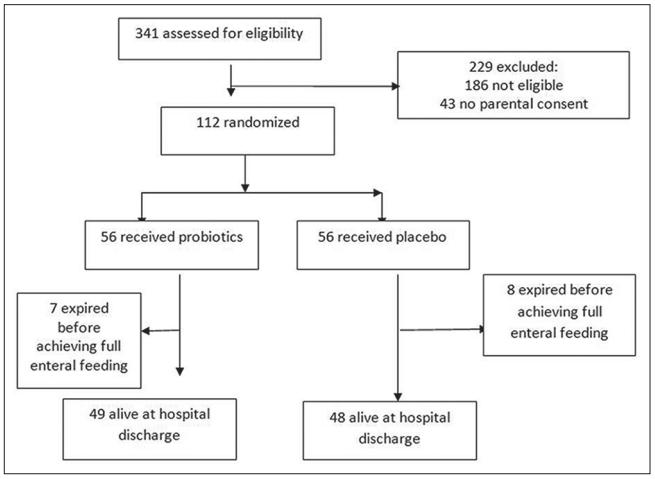

Figure 1: Flow of patients through the trial

The primary outcome is decreased fungal colonization in gastrointestinal tract (GIT) with probiotics. The secondary outcomes are: Incidence of late onset septicemia; NEC graded by modified Bell's criteria;[30] mortality; duration of the primary hospital admission; number of days until full enteral feeds established (120 ml/kg/day or more for 3 consecutive days).

The probiotic used was Prowel by Alkem Batch PWS3002C containing *Lactobacillus acidophilus* 1.25 billion, *B. longum* 0.125 billion, *B. bifidum* 0.125 billion, and *B. lactis* 1.0 billion per 1 g sachet.

For quality control and safety, study batch and placebo was tested for the presence of pathogens using standard microbiological techniques and for the presence and quantitation of the probiotic organisms.

The newborns were randomized into two groups by a random-generated (computer-generated), predetermined number table. Group I (probiotic group) (n = 56) received supplementation with probiotics daily from the first 72 h for 6 weeks or until discharged as long as minimal enteral nutrition was not contraindicated. Group II (control) (n = 56) received sterile water as placebo. The dose of 6 × 109 colony forming units (CFU)/day of lactobacillus, that is, half of 1 g sachet was chosen on the basis of published data from previous studies of VLBW neonates.[31] For ELBW and <32 weeks, the starting dose should be 1.5 × 109 CFU/day for ELBW neonates until they reach enteral feeds of 50–60 ml/kg/day when the dose was increased to 3 × 109 CFU/day.[32] Three hundred and forty-one assessed for eligibility. All doctors, nurses, laboratory staff, and parents are blind to the randomized allocation.

The study intervention/placebo is given orally or via gastric tube twice daily with expressed breast milk in infants receiving minimal enteral nutrition. Freshly expressed mother's breast milk is the feed of choice followed by frozen breast milk, if fresh is not available. Intravenous fluids and nutrition are used until approximately 120 ml/kg of milk is tolerated per 24 h period.

Antimycotic treatment consisted of liposomal amphotericin B at the initial dose of 1 mg kg/day, with a gradual increase up to a maximum of 6 mg kg/day.[33] Treatment was stopped 7 days after a negative culture for Candida and three consecutive negative C-reactive protein. Antibiotic treatment was carried out after antibiotic assays. Treatment was stopped 7–12 days after negative assay of C-reactive protein and the absence of clinical signs of infection.[34]

Clinical procedures

Within few hours after delivery, enteral nutrition was started with 1 ml of human milk given every 2 or 3 h, and the amount of milk given was increased by 1.0 ml every 3–6 h, as tolerated; human milk was supplemented with parenteral glucose administered from day 1 of life and with amino acids and lipids administered from day 2 of life through a Premicath catheter. Nutrition administered with intermittent meals was progressively increased, if tolerated, and parenteral nutrition was progressively decreased and stopped, following the same protocols as reported by the international guidelines.[35]

Infants were weighed daily and examined by doctors at least twice daily for gastrointestinal symptoms.[36] Such as regurgitation (defined as the passage of refluxed gastric contents into the oral pharynx), vomiting/feeding intolerance (defined as the expulsion of the refluxed gastric contents from the mouth), abdominal distension, and characteristics of the feces. Length of hospitalization was also recorded.

Clinical signs of infection were monitored, including fever, desaturation, apnea, bradycardia, pallor or cyanosis, necessity of oxygen supplementation, and intubation. The laboratory parameters monitored were C-reactive protein and blood count. Investigations to detect any mycotic involvement of the organs included ultrasounds (renal, cardiac, abdominal, and transfontanellar), examination of the fundus oculi, and chest X-rays. Cranial ultrasound was performed for each preterm infant in the 1st week of life and another between 15 and 21 days after birth and at least one at term age.

Microbiology

Gastric aspirations were cultured for Candida detection at birth and after 7, 14, 21, 28, 35, and 42 days; quantitative fungal stool cultures were also examined at the same time.[37] For fungal culture, 0.2 g of specimen was diluted with 1.8 ml of sterile saline. Ten microliter aliquot was then plated on Sabouraud's dextrose agar containing 300 μg/ml chloramphenicol and 10 μg/ml gentamicin and incubated in air at 35°C for 48 h. *C albicans* was identified by germtubes and chlamydospore formation. Species identification of germ tube negative yeasts was done by by commercial API C20 AUX yeast kit. Yeast counts were obtained by colony counting 48 h after incubation. Infants were considered at high level of colonization if they presented >10^4 CFU/g of feces.[38] Blood cultures and Platelia Candida test were conducted for the diagnosis of invasive candidiasis and to evaluate antifungal chemotherapy efficacy.[39]

Proven fungal or bacterial infection was defined as a positive culture: (1) From blood (drawn from peripheral sites); (2) from urine (collected by suprapubic sterile puncture or sterile bladder catheterization, with a growth of 10,000 fungal organisms per ml); (3) from cerebrospinal fluid; or (4) from intravascular catheter tip (only considered proof of microbiologically documented fungal infection in patients with previous peripheral colonization by the same species).

Statistical methods

Sample size calculation

To reduce the incidence of culture proven sepsis from 33% (from local epidemiological data) to 16% (a 51.5% reduction) with a power of 0.8, the estimated sample size is 83 per group. The trial will therefore recruit 163 infants over 1 year.

Intention-to-treat analysis

Data from all randomized participants will be considered in the intention-to-treat model (for the primary outcome).

The primary end point was to evaluate the incidence of enteric fungal colonization. Statistical Package for Social Sciences (SPSS) statistical software for Windows, version 17.0 (SPSS Inc.), was used for all statistical computations. Student's *t*-test or the Mann-Whitney U test, when appropriate, was used for comparison of continuous variables; and a Chi-square test or Fisher's exact test, when appropriate, was used for comparison of categorical variables. The anthropometric variables (weight at birth and gestational age) were reported as mean ± standard deviation (SD). The intergroup comparisons for all variables were performed by independent sample's *t*-test. All tests were two-tailed. The level of significance was set at $P < 0.05$.

Results

A total of 112 preterm neonates were enrolled, 56 were randomly assigned to the study group and 56 to the placebo group. The baseline demographic characteristics between the two groups are reported in Table 1 and no significant differences are found between the two groups.

Enteral feeding was started at a similar postnatal age in probiotic (9.96 ± 5.20d) and placebo (11.22 ± 5.04d) groups ($P = 0.2$) and consisted of human milk in all neonates in both the groups. Oral supplementation with probiotics or placebo started in parallel with enteral feeding: 52.14 ± 17.14 and 50.68 ± 16.8 h and there is no significant difference ($P > 0.05$). Feeding advancement

Table 1: Baseline demographic profile of the study patients of the two groups

Parameters	Probiotic group (*n*=56) (%)	Control group (*n*=56) (%)	*P* value (two-tailed)
Male	14 (50)	16 (57.1)	0.789
Birth weight (mean±SD) (g)	1192±341	1069±365	
Gestational age (mean±SD) (weeks)	32±2	32.2±2	0.921
SGA/IUGR	26 (46.4)	24 (42.9)	0.823
Maternal parameters			
Poor socioeconomic status	15 (26.8)	16 (28.6)	0.812
Diabetes/GDM	5 (8.9)	6 (10.7)	1
Received antenatal care	45 (80.4)	49 (87.5)	0.835
H/O PROM	12 (21.4)	19 (27.7)	0.205
Cesarean delivery	47 (83.9)	43 (76.8)	0.476
Pregnancy-induced hypertension	9 (19.6)	4 (8.7)	0.231
H/O maternal fever in last 5 days prior to delivery	5 (8.9)	2 (3.6)	0.438
Received antenatal steroids	47 (83.9)	45 (80.4)	0.806
Multiple births	5 (8.9)	5 (8.9)	1
Received antenatal antibiotics	24 (42.9)	31 (55.4)	0.257
Postnatal demographic profile of study population			
H/O perinatal asphyxia	16 (28.6)	12 (21.4)	0.513
Needed mechanical ventilation	5 (8.9)	6 (10.7)	1
Received H2 blockers/PPI	4 (7.4)	8 (14.8)	0.361
Central venous line placement	19 (33.9)	17 (30.4)	0.84
Abnormal cranial USG	13 (23.2)	15 (26.8)	0.828
Received steroids	15 (26.8)	15 (26.8)	1

SD: Standard deviation; SGA: Small for gestational age; IUGR: Intrauterine growth restriction; GDM: Gestational diabetes mellitus; H/O: History of; PROM: Premature rupture of membranes; PPI: Proton pump inhibtors; USG: Ultrasonography

in the probiotics group was done by 4.51 ± 3.0 ml daily and by 3.27 ± 2.0 ml in the control group (P = 0.012). Full feed establishment was in 11.22 ± 5.04 days in probiotics group compared to 15.41 ± 8.07 days in the placebo group (P = 0.016) which is significantly earlier in the probiotic group.

The total number of days on total parenteral nutrition in the probiotics group was 6.91 ± 5.9 days compared with 6.36 ± 5.30 days in the placebo group (P = 0.6). The duration of hospitalization was 25.77 ± 9.16 days in the probiotic group compared to 31.21 ± 12.67 days in the placebo group (P = 0.002).

Stool fungal colonization, one of the important outcome parameters was 3.03 ± 2.33 × 10^5 CFU in the probiotics group compared to 3 ± 1.5 × 10^5 CFU in the placebo group (P = 0.03).

The total leukocyte count (TLC) on day 3 of treatment in the probiotics group was 7090 ± 2148.006 cells/mm^3 compared to 6626.79 ± 1888.674 cells/mm^3 in the placebo group (P = 0.22). TLC on day 9 of treatment is 5553.52 ± 1854.58 cells/mm^3 in the probiotics group compared to 4277.36 ± 1376.68 cells/mm^3 in the placebo group (P = 0). TLC on day 15 of treatment is 6190.57 ± 1295 cells/mm^3 in the probiotics group compared to 5572.55 ± 1269.658 cells/mm^3 in the placebo group (P = 0.001). The C-reactive protein (CRP) on day 3 of treatment 0.773 ± 0.3498 mg/dl in the probiotics group compared to 0.784 ± 0.2682 mg/dl in the placebo group (P = 0.8). The CRP values on day 9 of treatment was 1.167 ± 0.4568 mg/dl in the probiotics group compared to 1.498 ± 0.4865 mg/dl in the placebo group (P = 0). The CRP values on day 15 of treatment in the probiotic group 0.742 ± 0.2635 mg/dl compared to 0.808 ± 0.29 mg/dl in the placebo group (P = 0.23).

Duration of antimycotic treatment in the probiotics group 7.59 ± 8.551 days compared to 13.5 ± 8.94 days in the placebo group (P = 0.001). Other significant outcome parameters are given in Table 2. The incidence of infections is significantly more in the placebo group than the probiotics group (P = 0.017).

The occurrence of late onset sepsis (inclusive of fungal sepsis) is 55.4% in the probiotics group and 75% in the control group (P = 0.02). There is no significant difference between the occurrence of gram negative and gram positive infections in the two groups.

There is absence of fungal sepsis in 58.9% in the probiotics group compared to absence of fungal infection in 25% of the control group (P = 0.001). 12.5% in the probiotics group had *Candida albicans* infection compared to 28.6% in the control group (P = 0.001%). None of the neonates in the probiotics group suffered from *C. glabrata* infection, while 35.7% of the neonates in the control group suffered from *C. glabrata* infection (P = 0.004). No significance differences were found between the two groups in colonization rates of *C. krusei* and *C. parapsilosis*. There are no significant differences in outcome parameters of death, NEC, third generation cepahalosporin requirement, vancomycin requirement, retinopathy of prematurity, etc.

There were no gastrointestinal symptoms in 71.4% of neonates in the probiotics group and no symptoms in 30.4% of neonates (P = 0.0001). Abdominal distension was present in 10.7% of neonates in the probiotics group and 37.5% of the neonates in the control group (P = 0.001), there were no significant differences between the groups in terms of vomiting and diarrhea. The neonates

Table 2: Outcome parameters

Parameters	Probiotic group (n=56) (%)	Control group (n=56) (%)	P value (two-tailed)
3rd gen cephalosporins required	23 (41.1)	27 (48.2)	0.569
Vancomycin required	14 (25)	18 (32.1)	0.531
Intubation for at least 1 day	16 (28.6)	21 (37.5)	0.452
Oxygen required for at least 2 days	36 (67.9)	40 (71.4)	0.577
Minor surgery	1 (1.8)	0	
NEC	2 (3.6)	2 (3.6)	
Culture negative	18 (32.1)	5 (9)	0.03
ROP	14 (25)	10 (17.9)	0.629
Death	7 (15.2)	8 (17.4)	0.5
Bacterial infections			
Gram positive	9 (16.1)	18 (32.1)	0.055
Gram negative	12 (21.4)	15 (26.8)	0.053
No infection	35 (62.5)	23 (41.1)	0.017
Fungal infections			
Candida albicans	7 (12.5)	16 (28.6)	0.001
Candida glabrata	0	20 (35.7)	0.004
Candida krusei	4 (7.1)	3 (5.4)	
Candida parapsilosis	4 (7.1)	2 (3.6)	
Rhodotorula	0	1 (1.8)	
No fungal infection	33 (58.9)	14 (25)	0.001
Feeding establishment	11.22±5.04 days	15.41±8.07 days	0.016
Duration of hospitalization	25.77±9.16 days	31.21±12.67 days	0.002
Stool fungal colonization	3.03±2.33×105 CFU	3±1.5×105 CFU	0.03
Duration of antimycotic treatment	7.59±8.551 days	13.5±8.94 days	0.001

NEC: Necrotizing enterocolitis; ROP: Retinopathy of prematurity; CFU: Colony forming units

Table 3: Outcome parameters in<1,000 g babies

Parameters	Probiotic group (*n*=11)	Control group (*n*=11)	*P* value
Stool fungal colonization	3.06±2.03×105 CFU	3±1.3×105 CFU	0.02
Feed advancement	3.51±3.0 ml	2.27±2.0 ml	0.013
Full feed establishment	13.22±5.04 days	17.41±8.07 days	0.014
Duration of hospitalization	28.78±9.16 days	34.21±11.68 days	0.004
NEC	1	1	>0.5
Fungal infection			
Candida	1	3	0.001
No fungal infection	6	2	0.001

NEC: Necrotizing enterocolitis; CFU: Colony forming units

under 1000 g are the most vulnerable and the outcome parameters concerning them is depicted in Table 3.

Discussion

The presence of fungal colonization at various sites of the gastrointestinal tract is a well-known risk factor for subsequent dissemination of fungal sepsis in preterm neonates.[10,40] To reduce the development of invasive fungal sepsis due to Candida species it will be helpful if the gut colonization is reduced.

At birth, intestinal colonization is derived from vaginal mucoses of the mother and fecal microflora. Diet can influence microflora and in breastfed neonates gut microflora is dominated by Bifidobacteria. Probiotics have wide ranged effects including modulation of gut microflora, promoting mucosal barrier functions, inhibiting mucosal pathogen adherence, and interacting with innate and adaptive immune systems of the host. The intestinal mucosal barrier consists of the intestinal microbiota that restrict mucosal colonization by pathogens and also resist penetration by pathogens. The direct effects of probiotics on the lumen are competition with the pathogens for nutrients, production of antimicrobial substances, receptorial hydrolysis, and nitric oxide (NO); while the indirect effects are based on site of interaction of the probiotics and the effectors of immune response topographically located in the intestinal tract.[41]

Premature neonates in the NICU are highly prone to develop disorders of gut microecology with an overgrowth of pathogenic microflora including fungi,[38] as they are often treated with long courses of antibiotics and also this group experience difficulty in receiving full enteral nutrition.[7,10-42] The gut is a reservoir site as well as a major colonization site for all types of pathogens and probably the most important site from where fungal dissemination occurs.[14-17]

Our study examined the effectiveness of probiotics (*Lactobacillus acidophilus* and *B. longum* in this case) in preventing the gastrointestinal colonization by Candida species in preterm low birth weight neonates.

The results show a significant reduction in gastrointestinal colonization by Candida species among low and VLBW preterm neonates given probiotics and gastrointestinal colonization being demonstrated by measuring stool fungal colonization. The presumed mechanisms by which the given probiotics modify the microecology including the fungal ecology in the gut are by competitive exclusion of fungi and the reduction in their ability to colonize the gut mucosa by increased mucosal IgA responses;[43] changes in intestinal permeability with an increased gut mucosal barrier to fungi and modifications of host response to fungi.[44,45] As in our study we used human milk for the neonates in both the groups, the results of the study are not attributable to the type of milk used. A study by Sims *et al*., described four-fold reduction in overall colonization of the gut by administering oral nystatin to preterm neonates with a birth weight of <1,250 g.[46] Studies by Kicklighter *et al*., and Kaufman *et al*., showed three-fold reduction in stool or rectal fungal colonization in neonates with birth weight of <1250 or 1000 g, respectively by administering prophylactic intravenous fluconazole.[17,18] However, the promising results obtained with fluconazole have to be weighed against the increased risk of emergence of resistant strains. Also the use of fluconazole as prophylactic treatment raises concerns about cost of the treatment.[26]

So strategies must be thought upon that reduce fungal colonization of gut with minimum cost and minimum adverse effects and minimum potential to cause emergence of drug resistant strains of fungi.

Our study shows the potential beneficial effects of probiotics on clinical and physiological variables related to gut function and probiotics administration in our study leads to earlier full feed establishment and increased rate of feeding advancement in the study group. These findings were corroborated by the findings from study by Rouge *et al*.,[47] Our study also showed significant reduction in the duration of hospital stay in the neonates belonging to the study group compared to the placebo group, which however is different from result by Rouge *et al*.,[47] but corroborated by the findings of Romeo *et al*.[1]

Our study showed that the incidence of infection is more in the placebo group than the probiotics group which is corroborated by studies by Lin *et al*., and Sims *et al*., and negated by findings of Rouge *et al*.[46-48] Our study shows that there is a significant increase in

fungal sepsis in the placebo group compared to the study group and significant increase in the infection by *C. albicans* and *C. glabrata* infection corroborated by findings of Romeo *et al*.[1]

So the key features of our study were reduced enteral fungal colonization, reduced invasive fungal sepsis, earlier establishment of full enteral feeds, and reduced duration of hospital stay in the study group. Probiotics had good safety profile and did not show any side effects in preterm neonates, furthermore their use reduced gastrointestinal symptoms in the study group. Limitations of our study are that we could not compare between the different types of probiotics and we did not follow-up the neonates neurologically on long-term basis.

Conclusion

To date only a few clinical trials have reported the outcomes for preterm neonates given probiotics. Although our findings regarding fungal colonization and prevention of IFI are encouraging larger, more definitive randomized control trials are required to establish or negate the use of probiotics as an additive treatment to prevent IFI in preterm neonates.

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