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Original Article

Paediatrics Section

Probiotics (*Bacillus clausii*) for Prevention of Late-onset Sepsis in Preterm Infants (<34 weeks): A Randomised Controlled Trial

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ABSTRACT

Introduction: Late-Onset Sepsis (LOS) causes significant morbidity and mortality in preterm infants. Probiotics have been suggested to improve the integrity of mucosal barrier by modifying the enteric microflora and suppress the overgrowth and translocation of pathogens in the gut, thus preventing life-threatening infections. Although probiotics have a definite role in prevention of Necrotising Enterocolitis (NEC) in preterm neonates, their effect on prevention of LOS in preterm neonates is still uncertain.

Aim: To evaluate the role of probiotics in reducing incidence of LOS in preterm neonates (<34 weeks).

Materials and Methods: A double blinded randomised control trial was conducted in a tertiary care Neonatal Intensive Care Unit (NICU) in Karnataka, India between 1st January 2019 to 31st December 2019. Seventy haemodynamically stable preterm neonates, <34 weeks of Gestational Age (GA), were randomised into 'Probiotic' and 'Placebo' group. The probiotic group (n=36) was prophylactically administered *Bacillus clausii* suspension at a dose of 2.5 mL per oral (0.4×10^9 spores in 1 mL) BD with breast milk, from initiation of enteral feeds till seven days, discharge/death/LOS, whichever was earlier. The placebo group (n=34) received breast milk with sterile water 2.5 mL per oral BD. All the neonates were investigated and managed as per standard hospital protocol. Primary outcome of the study was to find the incidence of LOS. Student's t-test, Mann-Whitney U test, Chi-square test and Fisher's-exact test were used for statistical analysis.

Results: There was no significant difference between the probiotic vs placebo group, with respect to incidence of LOS {(11.11% vs 17.7%); p-value >0.05} and duration of hospital stay {(10.86±3.19 vs 11.23±2.98 days); p-value >0.05}. However, incidence of feed intolerance in the probiotic group (11.11%) was significantly less than that the other (26.47%) (p<0.05).

Conclusion: Probiotics, prophylactically fed enterally, did not reduce the incidence of LOS but provide a promising strategy to prevent feed intolerance in premature neonates.

Keywords: Enteral nutrition, Gut microbiota, Mucosal immunity, Neonatal sepsis

INTRODUCTION

The incidence of Late-Onset Sepsis (LOS) in preterm infants is estimated to be 30 per 1000 live births [1]. Neonates, especially preterm infants are at higher risk of morbidity and mortality secondary to sepsis, because of developmental immaturity of the immune system, increased incidence of Caesarean delivery leading to inadequate colonisation of skin and mucosal surfaces by protective bacteria and ineffective barrier functioning of the preterm skin. Also, preterm neonates are more likely to be subjected to an increased use of invasive procedures and devices as well as broad-spectrum antibiotics [2]. It is crucial to prevent LOS, given the high risk of long-term neurodevelopmental disorders, even after its treatment [3].

Probiotics have been suggested to improve the integrity of mucosal barrier by promoting the growth of protective gut microbiota, suppressing the growth and translocation of pathogenic bacteria in the gut and producing protective factors like immunoglobulin-A and bacteriocins, thus preventing life-threatening neonatal sepsis [4]. Although probiotics have a definite role in prevention of stage II to III Necrotising Enterocolitis (NEC) in preterm neonates, their definite effect on prevention of LOS in preterm neonates is still controversial [5,6].

Tewari VV et al., used probiotic *Bacillus clausii*, and found no significant difference in the incidence of LOS between probiotic and placebo groups, whereas the study by Roy A et al., using a combination of probiotics (*Lactobacillus* and *Bifidobacterium* strains), showed decreased rates of stool fungal colonisation and LOS by *Candida* species [7,8]. The meta-analysis by Rao SC et al., pooled data from 37 randomised trials and concluded that probiotic supplementation reduces the risk of LOS in preterm infants [9].

In the studies done by Tewari VV et al., and Roy A et al., full feeds were achieved significantly faster in the probiotic group [7,8], similar to the findings by Samanta M et al., [10]. However, this was contrary to the findings of by Shashidhar A et al., who also did not find any difference for the duration of hospital stay between the two groups [11].

The aim of this study was to investigate whether enteric probiotic supplementation reduced the risk of LOS in preterm neonates in NICU. The primary outcome measure was incidence of LOS (probable and definite), and secondary outcome measures were incidence of feed intolerance and duration of hospital stay.

MATERIALS AND METHODS

This double blinded randomised controlled trial was conducted at NICU of a tertiary care referral hospital between 1st January 2019 and 31st December 2019. Institutional Ethics Committee (IEC) clearance was taken [IEC letter no (BIMS-IEC/41/2018-19)] and a written informed consent was taken from the parent/guardian before enrolment.

Sample size: The minimum sample size formula based on two proportions were used for 5% level of the significance and for 80% power of the test according to a study [8], the minimum sample size obtained was 33 in each group.

Inclusion criteria: Preterm neonates <34 weeks admitted to the NICU, who were haemodynamically stable and did not require Continuous Positive Airway Pressure (CPAP) or ventilator assistance on day 1 of life, were included in the study within 24 hours of birth.

Exclusion criteria: Extramural preterm neonates; sick preterm neonates; preterm neonates with an intestinal surgical anomaly, lethal congenital anomaly, dysmorphism or aneuploidy were excluded from the study.

Methods of Data Collection

Of the 70 neonates analysed, 36 neonates were randomised to the test group and 34 to the placebo group, on the basis of a computer generated random number table. Neonates in the test group received probiotics and were compared with those in the placebo group. Intervention was started with the trophic feeds within the first 24 hours of life. Probiotic group received suspension of *Bacillus clausii* in a dose of 2.5 mL per oral (0.4×10^9 spores in 1 mL) every 12 hours mixed with the enteral feeds through orogastric tube or oral feeds, giving them 2×10^9 spores per day. Probiotic used was *Bacillus clausii* suspension in mini bottles of 5 mL each, containing 2 billion spores, (Enteromax suspension; Mankind Pharma Pvt. Ltd.) stored at a temperature not exceeding 25°C according to the manufacturer's specifications. The placebo group received sterile water, 2.5 mL per oral every 12 hours mixed with feeds. Probiotic supplementation was continued till seven days/discharge/death/occurrence of LOS or NEC, whichever was earlier. Antibiotics were not started in both the groups.

Blinding: The investigators, doctors and nurses in NICU were all blinded to the intervention. All probiotic and sterile water mini bottles were coded and their labels were concealed. They were packed in serially numbered opaque sealed envelopes which were available with the in-charge nurse of the NICU. The in-charge nurse then administered the probiotic or placebo to the neonates orally as per allocation and unused portions of probiotic or placebo were discarded. The decoding of the allocation was done only after completion of analysis.

Feeding was started as per institutional feeding protocol when the neonate was haemodynamically stable, had normal vital signs, normal bowel sounds and no abdominal distension or altered aspirates from nasogastric tube. Depending on the birth weight and gestational age of the neonate, expressed breast milk was started at 20-30 mL/kg/day. Feedings were gradually increased, if tolerated, upto 150 mL/kg/day, with an increase of not more than 20 mL/kg/day. Standard management protocols of our NICU were followed for all these neonates.

Laboratory tests: Just before commencement of probiotic therapy, specimens of blood were obtained from all neonates for complete blood count, blood culture and sensitivity, arterial blood gas (as indicated) (on first day) and serum electrolytes and C-Reactive Protein (CRP) (on the third day). Chest X-ray was done in cases with signs of respiratory distress or in suspected pneumonia. Repeat blood culture and sepsis screen was done in all neonates clinically suspected to have sepsis.

Sepsis screen [12-15]: Two or more abnormal parameters were considered a positive screen [Table/Fig-1].

S. No.	Components	Abnormal value
1.	Total leukocyte count	<5000/mm^3
2.	Absolute neutrophil count	1800/mm^3
3.	Immature/Total neutrophil	>0.2
4.	Micro-Erythrocyte sedimentation rate	>15 mm in 1st hour
5.	C-Reactive Protein (CRP)	>1 mg/dL

[Table/Fig-1]: Sepsis screen.

Monitoring: During the period of hospitalisation, clinical examination of the neonates were performed daily based on a structured form. The vital parameters {including heart rate, respiratory rate, body temperature, colour, capillary refilling time, pulse oximetry, Non Invasive Blood Pressure (NIBP)} along with respiratory distress score (Silverman Anderson score), prefeed residual volume and abdominal girth were measured 6th hourly. Blood sugar was monitored by glucometer 12th hourly. Total time taken for attainment of full feeds (150 mL/kg/day) were noted. All neonates were fed with expressed breast milk.

The intervention was stopped if one or more of the following clinical features, along with two abnormal parameters on septic screen were present: Prefeed gastric aspirate or vomit that is bilious or contains blood; Respiratory distress, apnoea and gasping respiration; Metabolic disturbances-hypoglycaemia or hyperglycaemia; Capillary Refill Time (CRT) >5 second or hypotension needing inotropic support; Clinical coagulopathy like petechiae, purpurae, bleeding from injection sites, etc. [7].

All neonates suspected to have sepsis had a septic screen to corroborate the diagnosis and if two (or more) parameters were abnormal, it was considered as a positive screen. If any of the above clinical features were present then empirical antibiotic therapy was instituted and supportive treatment was given. Clinical suspicion along with positive sepsis screen was considered as probable sepsis. Neonate with growth on blood culture was labelled as having definite sepsis.

Feed intolerance [16]: Since there are no fixed criteria to define feed intolerance in preterm infants, the following clinical features were considered to be suggestive of feed intolerance: symptoms like vomiting, increased or altered (bilious or blood stained) gastric aspirates, systemic symptoms like lethargy, apnoea, poor feeding; and signs like distended or tender abdomen, reduced or absent bowel sounds and systemic signs like poor perfusion, poor tone and activity, cyanosis, bradycardia, etc.

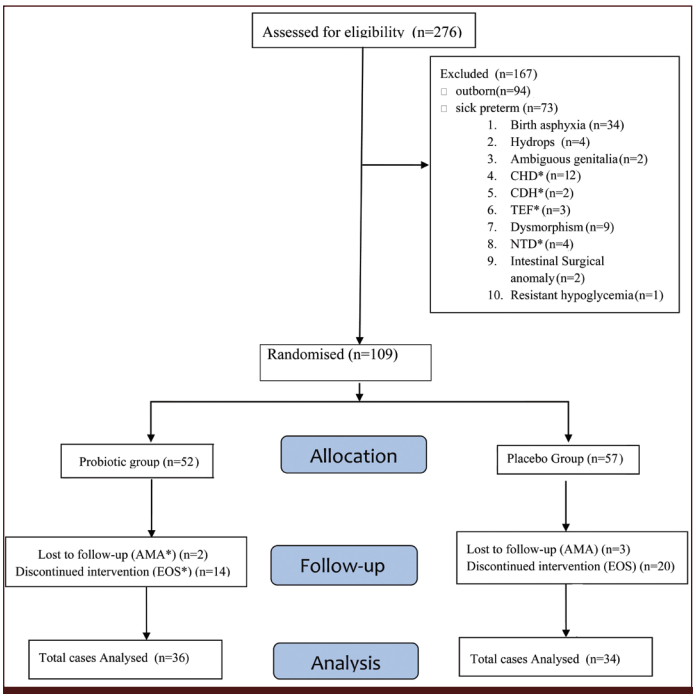
STATISTICAL ANALYSIS

The Student's t-test or the Mann-Whitney U test, wherever appropriate, were used to compare continuous variables, whereas Chi-square analysis or Fisher's-exact test were used to compare categorical variables between groups. The differences were considered statistically significant when p-value <0.05. Qualitative data was represented using frequencies and proportions. Statistical analysis was done using software package Statistical Package for the Social Sciences (SPSS) version 21.0. Graphical representation of data was done using MS Excel and MS Word.

RESULTS

[Table/Fig-2] shows the flowchart of study subjects through the phases of the study. There were 36 neonates in the probiotic group and 34 in the placebo group. Neonates in both the groups had

comparable baseline characteristics [Table/Fig-3]. Implementation of the intervention was similar in both the groups [Table/Fig-4].



[Table/Fig-2]: Flowchart of study subjects.
*CDH: Congenital diaphragmatic hernia; *CHD: Congenital heart disease; *TEF: Tracheo-esophageal fistula; NTD: Neural tube defects; *AMA: Discharge against medical advice; *EOS: Early onset sepsis

Parameters	Probiotic group (n=36) Mean±SD or Number (%)	Placebo group (n=34) Mean±SD or Number (%)	p-value
Birth weight, grams	1721.11±206.93	1774.70±142.15	0.354
Gestation, week	33.44±1.2	33.35±1.8	0.286
Male sex	17 (47.2)	18 (52.9)	0.782
Caesarean birth	21 (58.3)	17 (50)	0.256
Singleton gestation	32 (88.9)	29 (85.3)	0.456
Primigravida	13 (36.1)	12 (35.3)	0.334
Small for Gestational Age (SGA)	11 (30.5)	9 (26.5)	0.286
Antenatal factors			
Complete antenatal steroids	7 (19.4)	8 (23.5)	0.892
Maternal antibiotics –Intrapartum	16 (44.4)	10 (29.4)	0.384
Preeclampsia	8 (22.2)	6 (17.6)	0.216
Preterm premature rupture of membranes	2 (5.6)	3 (8.8)	0.322
Spontaneous onset of labor	22 (61.1)	23 (67.6)	0.456
Investigations			
Haemoglobin (G/L)	153.9±10	153.0±9.3	0.714
Total leukocyte count ($\times 10^9$/L)	11.71±3.25	13.18±4.57	0.128
Platelet count ($\times 10^9$/L)	216.88±22.79	181.36±35.31	0.920
Day 3 investigations			
Blood urea (mmol/L)	9.53±2	9.35±2.06	0.470
Serum creatinine (µmol/L)	62.36±20.52	55.45±18.85	0.153
Potassium (mmol/L)	4.77±0.75	4.79±0.66	0.902
Sodium (mmol/L)	139.11±4.21	140.27±3.63	0.288
CRP (mg/dL)	0.51±0.1	0.46±0.1	0.065

[Table/Fig-3]: Baseline characteristics of participants.

Variables	Probiotic group n (%)	Placebo group n (%)	Z Statistics	p-value
Intervention started at (In hour)				
Mean±SD (In hour) (Confidence interval for mean)	5.76±1.27 (5.33-6.19)	6.02±1.55 (5.48-6.56)	0.877	0.215
Intervention given till				
<7 days	4 (11.1)	6 (17.7)	0.78	0.194
≥7 days	32 (88.9)	28 (82.3)	0.78	0.592
Intervention discontinued due to				
Probable sepsis	2 (5.6)	5 (14.7)	1.28	0.194
Definite sepsis	2 (5.6)	1 (2.9)	0.54	

[Table/Fig-4]: Implementation of the intervention.

Intervention (probiotics in cases and sterile water in placebo group) in this study was started along with trophic feeds and it was initiated after the infant was stabilised. The mean time of starting intervention was 5.76±1.27 hours in the probiotic group whereas it was 6.02±1.55 hours in the placebo group. However, no significant difference was observed in time of initiation of intervention amongst probiotics and placebo (p>0.05) groups [Table/Fig-4].

Intervention was given for less than a week in 4/36 (11.1%) cases and for complete seven days in 32/36 (88.9%) cases in the probiotics group. In the placebo group, 6/34 (17.7%) newborns were given intervention for less than seven days while 28/34 (82.3%) newborns were given intervention till complete seven days. There was no significant difference between the two groups in terms of duration of intervention given [Table/Fig-5].

Characteristics	Probiotic group n (%)	Placebo group n (%)	p-value
Sepsis (Probable and definite sepsis)	4/36 (11.11)	6/34 (17.64)	0.194
Time taken to reach full feeds Mean±SD (in days) (Confidence interval)	7.5±2.63 (7.09-7.91)	8.99±1.21 (7.88-9.71)	0.008*
Feed intolerance n (%)	3/36 (11.11)	9/34 (26.47)	0.002*
Duration of hospital stay Mean±SD (in days)	10.86±3.19	11.23±2.98	0.978

[Table/Fig-5]: Outcome in preterms in probiotic and placebo groups.
*p<0.05 is significant

The intervention was discontinued before seven days in 2/36 (5.6%) and 5/34 (14.7%) in the probiotic and placebo groups respectively due to probable sepsis whereas in 2/36 (5.6%) and 1/34 (2.9%) newborns in probiotic and placebo groups respectively due to definite sepsis. There were no cases of NEC in both the groups. No significant difference was noted between probiotic group and controls with regards to premature discontinuation of intervention due to death, sepsis or NEC (p>0.05). There were no deaths recorded during the study.

The primary outcome of incidence of LOS (includes probable and definite sepsis) was 11.11% in the probiotic group and 17.64% in the placebo group, however the difference was not statistically significant (p>0.05). A secondary outcome was the incidence of feed intolerance which was 11.11% in the probiotic group and 26.47% in the placebo group. The incidence of feed intolerance was significantly less in the probiotic group (p-value=0.002). Also there was a significant difference in the time taken to reach full feeds between the probiotic and placebo groups (p-value=0.008). The neonates in the probiotic group took 7.5±2.63 days to attain full feeds while neonates in the placebo group took 8.99±1.21 days for the same.

Another secondary outcome was duration of hospital stay, which was not significantly different between probiotic and placebo groups (p>0.05). However, in this study, 32/36 (88.9%) neonates in the probiotic group and 26/34 (76.5%) neonates in the placebo group were hospitalised for less than two weeks. There were a significantly less number of neonates who stayed for more than two weeks in the probiotic group compared to the placebo group. No unexpected adverse events were observed during the course of the study.

DISCUSSION

Low Birth Weight (LBW) preterm neonates are at increased risk of intestinal colonisation with pathogenic bacteria which trigger inflammatory processes and lead to LOS and NEC. Exposure to antibiotics after birth and prolonged hospitalisation reduce the diversity of this beneficial gut microbiome [17].

This study compared the use of probiotics versus placebo in prevention of LOS. There was no significant reduction in the incidence of LOS (4 of 36 neonates-11.1% vs 6 of 34 neonates-17.6%; p-value >0.05).However, the time taken to reach full feeds was significantly less in the probiotics group (7.5±2.63 days vs 8.99±1.21 days; p-value <0.05), with a decreased incidence of feed intolerance (3 of 36 neonates - 11.11% vs 9 of 34 neonates - 26.47%; p-value <0.05). The present study demonstrated the safety and tolerance of *B.clausii* in preterms. In this study, however, there was no significant difference between the two groups in terms of duration of hospital stay (10.86±3.19 days vs 11.23±2.98 days; p-value >0.05).

The choice to study *Bacillus clausii* was made as this spore forming bacteria which is safe and approved for breastfed neonates, is heat stable, can be stored at room temperature without any loss of viability. Also, it is resistant to acidic conditions of the stomach, accurate dosing is feasible and the availability of liquid formulation obviates issues of reconstitution [18].

Similar to the present study, Tewari VV et al., using 2.4×10^9 spores of *Bacillus clausii* per day, reported no significant difference in the incidence of LOS between probiotic and placebo groups (p-value=0.36 and 0.32 in extreme preterm and very preterm groups respectively), although 90% in extreme preterm and 66% in very preterm groups received probiotic for ≥4 weeks [7]. Contrary to this, the meta-analysis by Rao SC et al., data from 37 RCT's, concluded that probiotic supplementation reduces the risk of LOS in preterms. However, in this meta-analysis a single strain probiotic was used in 23 studies, whereas 14 used multiple strains; most studies used *Lactobacillus*, *Bifidobacterium* or *Saccharomyces* strains; only two studies used *B. clausii* [9].

In this study time taken to reach full feeds was also significantly less in the probiotics group, with decreased risk of feed intolerance as compared to placebo group. Similar findings were observed in the study done by Tewari VV et al., in which full feeds were achieved significantly faster in the probiotic group, in both extreme preterm and very preterm neonates [7]. These findings are also supported by the meta-analysis done by Roy A et al., as well as studies done by Samanta M et al., and Athalye-Jape G et al., [8,10,19].

Contrarily, the study done by Shashidhar A et al., using a multicomponent probiotic formulation did not find significant improvement of feed tolerance in very low birth weight neonates, probable reasons being predominant use of breast milk in all neonates and significantly higher number of Caesarean deliveries in the no probiotic group [11].

Indrio F et al., also studied stool cytokine levels which were significantly reduced in preterm infants receiving probiotics along with reduced incidence of feed intolerance [20]. The results of a recent meta-analysis by Zhang W et al., also showed a beneficial effect of probiotics on preterm infants with regard to total amount of feeds reached, weight gain, and duration of hospital stay with better feed tolerance [21].

The present study didn't differ between the two arms in terms of duration of hospital stay, similar to the findings of the study done by Tewari VV et al., [7]. Unlike in the studies done by Samanta et al., and Roy et al., using probiotic combinations the duration of hospital stay was significantly less in the probiotic group [8,10]. The probable reasons for the difference in the findings could be due to the different probiotic strains used, use of multistrain preparations with different dosing and duration protocols.

The strength of the study was its double blinded design, use of single strain probiotic and avoidance of prophylactic antibiotics to all the study neonates.

Limitation(s)

A single dosage of Bacillus clausii, was used. The study lacked a follow-up of the participants.

CONCLUSION(S)

In conclusion, in this study prophylactic administration of *Bacillus clausii* had no significant effect on the incidence of LOS in preterm neonates <34 weeks, however showed significantly faster attainment of full feeds and reduced incidence of feed intolerance in premature neonates.

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