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# Role of Lactobacillus Rhamnosus GG in Prevention of Necrotizing Entrocolitis and Late Onset Sepsis in Preterm Neonates < 35 Weeks: A Randomized Controlled Trial

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## ABSTRACT

***Background:*** There is a paucity of RCTs that have systemically evaluated the effects of L.GG on the prevention of NEC and LOS in preterm neonates.
To study different incidence rates of NEC and LOS. Secondary outcomes were the incidence of hyperbillirubinaemia, mortality, side effects and weight gain.
***Methods:*** This is an open labeled RCT. Preterm infants with a gestational age of < 35 weeks were assigned to three groups: A(L.GG sachet 6 billion organism), B(L.GG drops 2 billion organisms), C (no probiotic) using fixed block randomization. Probiotic was administered till the neonate reached the corrected gestational age of 36 weeks or a maximum of 4 weeks.
***Results:*** Of 123 neonates, NEC developed in 2(4.88%) in group A versus 1(2.38%) in group B and 0 (0%) in group C (p value- 0.37). LOS also developed in 5(12.2%) in group A versus 3(7.14%) in group B and 3(7.5%) in group C (p-value 0.7). Moreover, 92.68% of subjects were successfully discharged in group A, 95.24% in group B and 90% in group C ( p-value 0.55). A significant difference was found between the incidence of hyperbilirubinemia (21.95% in group A versus 47.5% in group C (p-value 0.02) and 28.92% in Group A+B versus 47.5% in group C (p-value 0.04). There was a significant difference in weight gain at 1 month of age; 9.65±3.72 grams/kg/day in group A versus 6.58±3.86 grams/kg/day in group C (p-value 0.002) and 8.98±4.49 grams/kg/day in group B versus 6.58±3.86 grams/kg/day in group C (p-value 0.03).
***Conclusion:*** L.GG alone as a single strain administered in both high and low dosages has no significant effect on reducing the incidence of NEC, LOS and yielding immediate outcomes. A larger sample size and a blinded study are required to draw more accurate conclusions.

***Keywords:*** L. GG, ,LOS, NEC, Prematurity

## Introduction

The etiology of NEC is multi-factorial. Immature intestinal function, formula feeding, bacterial dysbiosis, and a hyper inflammatory host response are key factors in the typical NEC of the preterm infant (1).

The administration of probiotic microorganisms to preterm newborns has recently piqued the public's curiosity. Probiotic bacteria, such as Bifidobacterium and Lactobacillus, are live microbial supplements that colonize the infant's intestines and prevent the growth of harmful organisms linked with NEC. Probiotics also boost gut barrier function and modify the local immune response. Probiotics have also been shown to

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increase gastrointestinal motility by reducing phase-3 intervals in migrating motor complexes. Probiotics have been shown to raise the villus height to the crypt depth ratio in the duodenum and ileum, resulting in a considerable surge in intestinal absorptive area, which facilitates the passage of digested nutrients into the villi (2).

There is a growing body of RCTs and observational research studies on probiotics. They almost uniformly agree that using probiotics in preterm newborns is safe and practical. Recent meta-analyses of RCTs state that the administration of probiotics in preterm newborns is associated with a statistically significant drop in the incidence of Necrotizing enterocolitis, late-onset sepsis, and death (3-6).

Till date protocols regarding the useful strains of probiotics strains and their optimal dose are not well established and it is not clear whether single probiotics is better than a combination of probiotics. There are countless of probiotic strains are available in the market singly with a variety of combinations; but few direct individual studies have been conducted for their comparison. Recently Chris et al. and Beghetti et al. did a network meta-analysis to give ranking to the various probiotics used. Authors of both network meta-analyses reported that most probiotic strains are understudied and that too in small experimental samples. Thus in the absence of significant effects of a particular probiotic, it is difficult to decide whether it is due to a lack of a randomized controlled trials or due to a lack of genuine efficacy. Hence, further trials are warranted to confirm the findings (7, 8).

Literature review suggests that paucity of randomized controlled trials that have systematically evaluated the effects of Lactobacillus rhamnosus GG in preterm neonates. We found only 2 randomized controlled trials and 3 observational studies wherein Lactobacillus rhamnosus had been used as a single strain. Both randomized controlled trials –conducted by Dani et al. & by Manzoni et al. used a higher daily dose of Lactobacillus rhamnosus (6 billion CFU) in VLBW neonates and they did not show any significant beneficial effects in the reduction of NEC & LOS(9,10). Similarly, retrospective observation study by Luoto et al., who utilized a similar dose of Lactobacillus GG in VLBW neonates; did not reflect any positive effects(11). In a retrospective observational cohort study by Kane et al. on VLBW neonates with variable doses of L. GG between 2.5 to 5 CFU/day, there was no decrease in rate of NEC was observed (12). The retrospective observational study by Bonsante et al. who used a low dose of Lactobacillus rhamnosus( 0.4 billion CFU) in very & extreme preterms below 31 weeks its revealed beneficial effects on the reduction of NEC and LOS (13).Therefore, this randomized controlled trial was conducted to determine positive effect of Lactobacillus rhamnosus GG in low (2 billion CFU) and high (6 billion CFU) doses in the prevention of NEC & LOS in preterm infants.

## Methods

### *Study Design & Setting*

This is an open labeled superiority randomized controlled trial that compares the high dose L. GG with a low dose of L. GG with no probiotic groups in terms of prevention of NEC and LOS in preterm. This study was conducted in NICU, Dhiraj hospital, Vadodara district, India from January 2000 to June 2021. The study was initiated after obtaining approval from the institutional ethics committee.

### *Inclusion & Exclusion Criteria*

Preterm neonates with a gestational age of < 35 weeks, both inborn neonates and out born neonates admitted within the first 24 hours of life were included in this study. Neonates with gestational age ≥ 35 weeks, out born neonates admitted after 24 hours of life, neonates with significant cardio-respiratory illness, neonates with gastrointestinal malformations, neonates in whom one is not able to start feed within 72 hours of birth and neonates whose parents were not willing to give consent for the study were excluded.

Neonates who met the inclusion criteria were included in the trial after obtaining informed parental consent.

### *Randomization, Allocation Concealment & Blinding*

Eligible neonates were randomly allocated to one of the three groups – group A (L GG in a higher dose of 6 billion CFUs daily), group B (L GG in a lower dose of 2 billion CFUs daily) and group C (no probiotic) by computer generated randomization sequence with a block size of 15 by an independent researcher. The allocation sequence was concealed in sealed opaque envelopes which were sequentially numbered by another independent staff member and were kept in the neonatal intensive care unit (NICU). The NICU nurse in charge on duty opened the sealed opaque envelope and disclosed the intervention.

Blinding was not ensured in this study.

***Intervention***

Neonates in Group A received probiotic sachet Superflora GG containing lyophilized Lactobacillus rhamnosus GG once a day (a total of 6 billion organisms daily)(Sundyota Numandis Pharmaceuticals) mixed with feed (a high dose of Lactobacillus rhamnosus GG group). Neonates in group B received probiotic drops Superflora GG containing lyophilized Lactobacillus rhamnosus GG 0.25 ml twice a day (total 2 billion organisms daily) (Sundyota Numandis Pharmaceuticals) mixed with a feed (low dose of Lactobacillus rhamnosus GG group) and neonates in group C did not receive any probiotic (control group). Probiotic was started with the introduction of feed and continued until the neonate reached the corrected gestational age of 36 weeks or a maximum of 4 weeks.

***Primary and Secondary Outcome Measures***

The primary outcome measures were to study differences in the incidence of necrotizing enterocolitis and the late onset sepsis. Secondary outcome measures involved the analysis of time required to reach full enteral feeds (110 ml/kg/day), the incidence of neonatal hyperbilirubinemia, duration of hospital stay,mortality rate, any untoward side effects and weight gain at one month of age.

***Monitoring & Follow up***

Apart from probiotic L. GG, each neonate was monitored and received care as per standard neonatal management protocols relative to their health status. Investigations were conducted as required.

The diagnosis of NEC was made based on modified Bell's staging which included systemic signs like temperature instability, lethargy, bradycardia, apnea, thrombocytopenia, acidosis, shock as well as intestinal signs like abdominal distention, absent bowel sounds, abdominal wall edema, abdominal wall induration together with radiological signs such as intestinal pneumatosis, portal vein gas with/without ascite and pneumoperitoneum (14).

The diagnosis of LOS was made based on start of sepsis after 72 hours of life. If the neonate had recently developed clinical features of sepsis like lethargy, poor activity, refusal to feed, apneic spells, hypoglycemia, hyperglycemia, poor perfusion, shock, respiratory distress, etc. not explained by any other conditions; then diagnosis of clinical sepsis was made. In neonates with clinical sepsis, blood culture and septic screen (TLC, ANC, IT ratio, CRP) were performed. If the septic screen was positive, the suspected sepsis was diagnosed; and if the culture was positive, the diagnosis of culture proven sepsis was made (15).

All the neonates were monitored regularly during their hospital stay. Neonates were discharged as per NICU policy and were followed up weekly until one month of age. At each visit; assessment of general well-being, assessment of weight and evaluation for signs/symptoms of sepsis / NEC were assessed.

***Sample size calculation***

The sample size was calculated based on the prevalence of LOS & NEC in the set up. Recent data indicated 12% prevalence of LOS in preterm neonates <35 weeks. Assuming 80% reduction in the frequency of LOS with Lactobacillus rhamnosus GG supplementation; with a significance level of 0.05 and power of 80% -a sample size of n= 113 was estimated for each group. For NEC, having 4% prevalence in preterm neonates <35 weeks; assuming an 80% reduction in frequency of NEC with Lactobacillus rhamnosus GG supplementation; with a significance level of 0.05 & power of 80%-a sample size of n=358 was calculated for each group.

This study was started in January 2020. Given the slow recruitment of patients probably due to COVID pandemic and time constraints, an interim analysis was performed on data until June 2021.

***Statistical Methods***

The data was primarily gathered in the structured Proforma and details were entered in an excel sheet. For all categorical variables the percentage was calculated. For continuous variables mean and standard deviation was calculated. For the comparison of baseline characteristics; the Chi square test was used for the analysis and comparison of categorical data.The student's t test or one way ANOVA test was used for analysis and comparison of continuous data. To compare antenatal and postnatal risk factors, a one sample chi-square test or goodness of fit test (McNemar chi-square test) was used. Primary outcomes (the incidence of NEC and the incidence of LOS) were analyzed by intention to treat analysis. For the analysis of primary outcomes; the chi square test was used. Among secondary outcomes; age to reach full feeds, length of hospital stay, time to regain birth weight, and weight gain at one month of age-

were analyzed using the protocol analysis. To investigate various secondary outcomes, the Chi square test was used for categorical data, and the student's t -test or one way- ANOVA test was used to analyze continuous data. All the statistical analysis was performed using SPSS software 20.0 version. All p values were two tailed and a p value of < 0.05 was considered as statistically significant.

## Results

Between January 2020 and June 2021, 148 preterm neonates of less than 35 weeks of gestational age were screened for eligibility; out of whom 123 were recruited and randomized in three groups of A, B & C. 41 neonates received Lactobacillus rhamnosus GG 6 billion CFUs daily (group A).Also, 42 neonates received Lactobacillus rhamnosus GG 2 billion CFUs daily (group B). In the 40 neonates in control group did not receive any drug (group C). All neonates were followed for primary outcome analyses. The trial flow diagram is depicted in Figure 1.

Baseline characteristics of 3 groups were compared; except for neonates with relatively larger birth weight neonates in the probiotic groups both group A & B (Table 1).

The presence of various antenatal and postnatal risk factors that can predispose neonates to sepsis and NEC were analyzed. They were almost identical but the number of mothers with PIH / eclampsia was slightly higher in group B & group C (Table 2 & 3).

The following statistical findings were observed regarding various primary and secondary outcome measures (Table 4, 5).

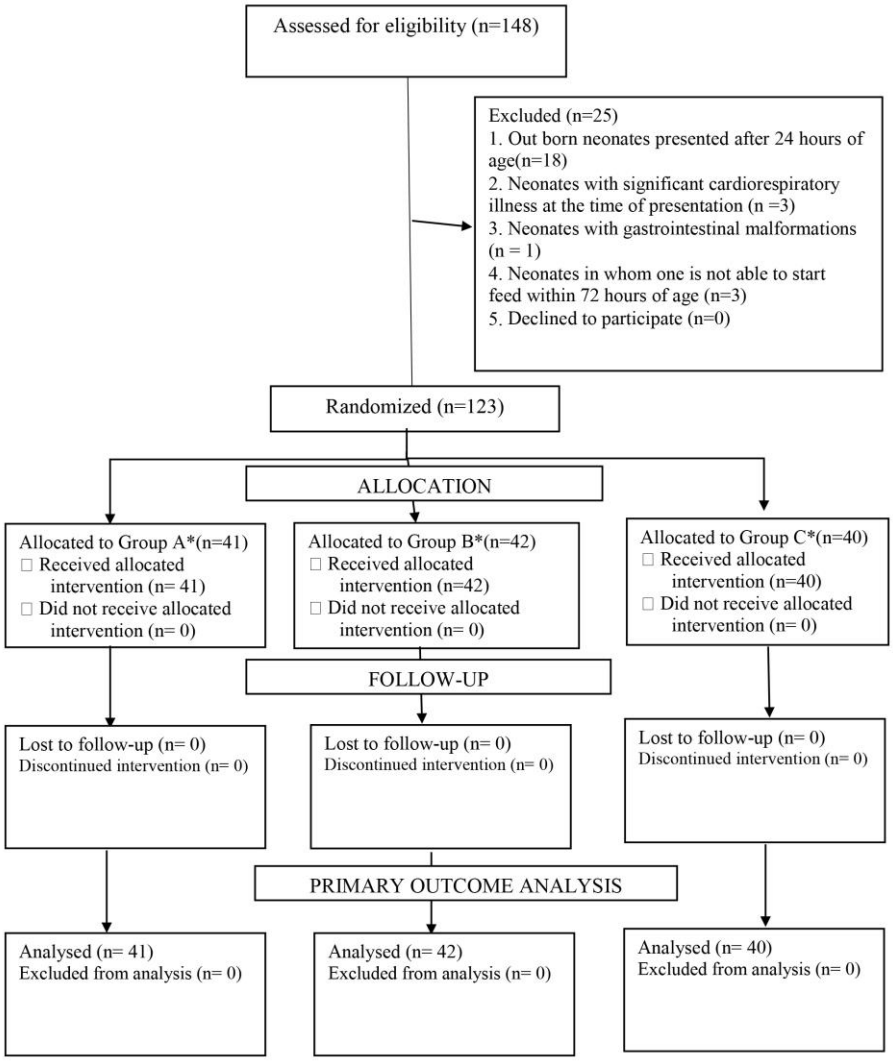


**Figure1.** Consort Flow Diagram

**Table 1**. Baseline Characteristics

| | Group A (N=41) | Group B (N=42) | Group C (N=40) | P-Value |
|---|---|---|---|---|
| Place Of Delivery | | | | |
| Intramural | 34 (82.93%) | 36 (85.71%) | 31 (77.50%) | |
| Extramural | 7 (17.07%) | 6 (14.29%) | 9 (22.50%) | 0.62 |
| Mode Of Delivery | | | | |
| Vaginal Delivery | 26(63.41%) | 19(45.24%) | 24(60.00%) | |
| Caesarean Section | 15(36.59%) | 23(54.76%) | 16(40.00%) | 0.21 |
| Gestational Age(Weeks) | | | | |
| <28 | 0(0.00%) | 0(0.00%) | 0(0.00%) | |
| 28 To<32 | 13(31.71%) | 15(35.71%) | 15(37.50%) | |
| 32 To <34 | 16(39.02%) | 13(30.95%) | 17(42.50%) | 0.67 |
| 34 To <35 | 12(29.27%) | 14(33.33%) | 8(20.00%) | |
| Mean ± Sd | 32.20 ± 1.82 | 32.29 ± 1.6 | 31.78 ± 1.75 | 0.37 |
| | 32.20 ± 1.82 | | 31.78 ± 1.75 | 0.29 |
| | | 32.29 ± 1.6 | 31.78 ± 1.75 | 0.17 |
| | 32.23 ± 1.71 | | 31.78 ± 1.75 | 0.18 |
| Birth Weight (Grams) | | | | |
| <1000 | 2 (4.88%) | 1 (2.38%) | 5 (12.50%) | |
| 1000-1499 | 12 (29.27%) | 15 (35.71%) | 17 (42.50%) | 0.19 |
| ≥1500 | 27 (65.85%) | 26 (61.90%) | 18 (45.00%) | |
| Mean ± Sd | 1578.78±302.13 | 1549.45±286.89 | 1435.75±344.02 | 0.1 |
| | 1578.78±302.13 | | 1435.75±344.02 | 0.05 |
| | | 1549.45±286.89 | 1435.75±344.02 | 0.11 |
| | 1563.94±293 | | 1435.75±344.02 | 0.03 |
| Weight For Gestational Age | | | | |
| AGA | 37(90.24%) | 35(83.33%) | 30(75.00%) | |
| SGA | 4(9.76%) | 7(16.67%) | 10(25.00%) | 0.31 |
| LGA | 0(0.00%) | 0(0.00%) | 0(0.00%) | |
| Sex | | | | |
| Male | 25 (60.98%) | 18 (42.86%) | 24 (60.00%) | |
| Female | 16 (39.02%) | 24 (57.14%) | 16 (40.00%) | 0.18 |
| Age Of Starting Of Feeding | | | | |
| < 24 Hours | 25(60.98%) | 30(71.43%) | 18(45.00%) | |
| 24 – 48 Hours | 13(31.71%) | 9(21.43%) | 18(45.00%) | 0.18 |
| 48 – 72 Hours | 3(7.32%) | 3(7.14%) | 4(10.00%) | |
| Type Of Milk Fed | | | | |
| Exclusive Breast Milk | 28(68.29%) | 28(66.67%) | 30(75.00%) | |
| Mixed | 13(31.71%) | 14(33.33%) | 10(25.00%) | 0.69 |

**Table 2.** Antenatal Risk Factors

| Risk Factor | Group A(N=41) | Group B(N=42) | Group C(N=40) | P Value |
|---|---|---|---|---|
| PPROM | 2(4.88%) | 3(7.14%) | 5(12.50%) | 0.5 |
| Perinatal Asphyxia | 2(4.88%) | 0(0.00%) | 4(10.00%) | 0.14 |
| Antenatal Steroids | 25(60.98%) | 20(47.62%) | 17(42.50%) | 0.46 |
| APH | 4(9.76%) | 1(2.38%) | 3(7.50%) | 0.44 |
| PIH/PE/Eclampsia | 0(0.00%) | 9(21.43%) | 6(15.00%) | 0.02 |
| GDM | 0(0.00%) | 0(0.00%) | 0(0.00%) | |
| Reverse/Absent End Diastolic Umbilical Arterial Flow (Doppler) | 1(2.44%) | 2(4.76%) | 2(5.00%) | 0.8 |
| Unsterile Cord Cut | 0(0.00%) | 1(2.38%) | 0(0.00%) | 0.36 |
| Maternal Fever | 0(0.00%) | 0(0.00%) | 0(0.00%) | |
| Prolonged Rupture Of Membrane (≥24 Hours) | 1(2.44%) | 3(7.14%) | 3(7.50%) | 0.56 |
| Oligo/Polyhydramnios | 4(9.76%) | 4(9.52%) | 4(10.00%) | 1 |

### *Incidence of NEC*

The incidence of NEC was 4.88% in group A, 2.38% in group B and 0% in group C; and there was no statistically significant difference in two groups (p value 0.37). The difference in incidence of NEC was also not significant between group A and C (p value 0.16), between group B and C (p value 0.33), and between group A+B combined and group C (p value 0.23)

### *Incidence of LOS*

The incidence of LOS was 12.2% in group A, 7.14% in group B and 7.5% in group C; there was no statistically significant difference in two groups (p value 0.7). It was also not significant between group A and C (p value 0.48), group B and C (p

**Table 3.** Presence Of Postnatal Risk Factors For Sepsis & Nec

| | Group A (N=41) | Group B (N=42) | Group C (N=40) | P Value |
|---|---|---|---|---|
| Parenteral Nutrition | 1(2.44%) | 1(2.38%) | 4(10.00%) | 0.22 |
| Intravenous Fluids > 7 Days | 4(9.76%) | 3(7.14%) | 5(12.50%) | 0.78 |
| Umbilical Venous Line | 7(17.07%) | 6(14.29%) | 10(25.00%) | 0.57 |
| Surfactant | 10(24.39%) | 4(9.52%) | 7(17.50%) | 0.28 |
| Invasive Ventilation (With / Without Non-Invasive Ventilation) | 8(19.51%) | 5(11.90%) | 11(27.50%) | 0.32 |
| Non-Invasive Ventilation | 11(26.83%) | 10(23.81%) | 11(27.50%) | 0.97 |
| CVL | 0(0.00%) | 0(0.00%) | 0(0.00%) | |
| ICD Tube | 0(0.00%) | 0(0.00%) | 0(0.00%) | |
| Urinary Catheter | 0(0.00%) | 0(0.00%) | 0(0.00%) | |
| Surgery | 2(4.88%) | 0(0.00%) | 2(5.00%) | 0.37 |
| Use Of Steroids | 0(0.00%) | 1(2.38%) | 0(0.00%) | 0.36 |
| Use Of H2 Blockers | 0(0.00%) | 0(0.00%) | 0(0.00%) | |

**Table 4.** Primary Outcome Measures

| Morbidities | Group A(N=41) | Group B(N=42) | Group C(N=40) | P-Value |
|---|---|---|---|---|
| NEC | 2(4.88%) | 1(2.38%) | 0(0.00%) | 0.37 |
| Group A Vs. Group C | 2(4.88%) | - | 0(0.00%) | 0.16 |
| Group B Vs. Group C | - | 1(2.38%) | 0(0.00%) | 0.33 |
| Group A+B Vs. Group C | 3 (3.6%) | | 0(0.00%) | 0.23 |
| LOS | 5(12.20%) | 3(7.14%) | 3(7.50%) | 0.7 |
| Group A Vs. Group C | 5(12.20%) | - | 3(7.50%) | 0.48 |
| Group B Vs. Group C | - | 3(7.14%) | 3(7.50%) | 0.95 |
| Group A+B Vs. Group C | 8 (9.6%) | | 3(7.50%) | 0.70 |

value 0.95), and combined group A+B and group C (p value 0.7).

### *Immediate Outcome*

No significant difference in immediate outcome. The rate of discharge was 92.68% in group A, 95.24% in group B and 90% in group C.

### *Incidence of Hyperbilirubinemia*

The incidence of hyperbilirubinemia was significantly lower in group A and in group A+B combined compared to group C (21.95% vs. 47.5%, p value 0.02 & 28.9% vs. 47.5%, p-value 0.04 respectively).

### *Feed Intolerance*

The incidence of feed intolerance was slightly higher in group A; though it was not statistically significant (14.63% Vs. 0% Vs. 7.5%).

### *Age to reach Full Feeds (110 ml / kg / day)*

All the neonates were able to reach full feeds of 110 ml / kg / day except one neonate in group C who never reached full feeds because of sickness. There was no statistically significant difference among 3 groups in age to reach full feeds. Neonates in group B reached full feeds earlier than those in group C (4.19 ± 1.74 vs. 5.08 ± 2.07 days, p value 0.04), but neonates in group A who received higher dose of L. GG did not reach full feeds earlier.

### *Duration of Hospital Stay for Discharged Neonates*

We were able to discharge 38 neonates in group A, 40 neonates in group B and 36 neonates in group C. The length of hospital stay was significantly lower in group A than in group C (14.11 ± 10.95 Vs. 20.38 ± 14.6 days, p value 0.04).

### *Time to Reach Birth Weight*

Newborn in group A was lost to follow - up after discharge, and therefore we could not assess time to reach birth weight in that neonate. There was a statistically significant difference in time to reach birth weight between groups (11.11±5.42 Vs. 11.73±5.29 Vs. 15.36±8.02 days, p value 0.01). Significant differences were observed between group A and group C, and between group B and group C.

### *Weight Gain (gram / kg / day at 1 month of age)*

A total of 6 neonates in group A, 5 in group B and 6 in group C were lost to follow up at one month of age after discharge; And was not possible to asses weight gain at 1 month of age. Through phone follow up it turned out that they were all alive and not sick. There was statistically significant difference in weight gain checked at 1 month of life (9.65±3.72 vs. 8.98±4.49 Vs. 6.58±3.86 gram / kg / day, p value 0.01). A significant difference was observed between group A and group C, and between group B and group C.

**Table 5.** Secondary Outcome Measures

| Morbidities | Group A(N=41) | Group B(N=42) | Group C(N=40) | P-Value |
|---|---|---|---|---|
| Outcome | | | | |
| Discharge | 38(92.68%) | 40(95.24%) | 36(90.00%) | |
| LAMA – Non-Moribund | 2(4.88%) | 0(0.00%) | 3(7.50%) | 0.55 |
| LAMA –Moribund | 1(2.44%) | 1(2.38%) | 0(0.00%) | |
| Death | 0(0.00%) | 1(2.38%) | 1(2.50%) | |
| Hyperbilirubinemia | 9(21.95%) | 15(35.71%) | 19(47.50%) | 0.17 |
| Group A Vs. Group C | 9(21.95%) | - | 19(47.50%) | 0.02 |
| Group B Vs. Group C | - | 15(35.71%) | 19(47.50%) | 0.28 |
| Group A+B Vs. Group C | 24 (28.9%) | | 19(47.50%) | 0.04 |
| Feed Intolerance | 6(14.63%) | 0(0.00%) | 3(7.50%) | 0.05 |
| Group A Vs. Group C | 6(14.63%) | - | 3(7.50%) | 0.31 |
| Group B Vs. Group C | - | 0(0.00%) | 3(7.50%) | 0.07 |
| Group A+B Vs. Group C | 6 (7.2%) | | 3(7.50%) | 0.95 |
| Age To Reach Full Feeds (110ml/Kg/Day) (DOL) | Group A(N=41) | Group B(N=42) | Group C(N=39) | |
| 3 | 12(29.27%) | 18(42.86%) | 5(12.82%) | |
| 4 | 12(29.27%) | 14(33.33%) | 17(43.59%) | |
| 5 | 5(12.20%) | 3(7.14%) | 6(15.38%) | |
| 6 | 7(17.07%) | 4(9.52%) | 5(12.82%) | 0.20 |
| ≥7 | 5(12.20%) | 3(7.14%) | 6(15.38%) | |
| Mean ± Sd | 4.61 ± 1.56 | 4.19 ± 1.74 | 5.08 ± 2.07 | 0.09 |
| | 4.61 ± 1.56 | | 5.08 ± 2.07 | 0.23 |
| | | 4.19 ± 1.74 | 5.08 ± 2.07 | 0.04 |
| | 4.39 ± 1.66 | | 5.08 ± 2.07 | 0.05 |
| Duration Of Hospital Stay (Days) Of Discharged Neonates | Group A (38) | Group B (40) | Group C (36) | |
| 1-3 | 0 (0.00%) | 0(0.00%) | 0(0.00%) | |
| 4-7 | 10(26.32%) | 9(22.5%) | 5(13.89%) | |
| 8-14 | 15(39.47%) | 12(30%) | 9(25%) | 0.24 |
| >14 | 13(34.21%) | 19(47.5%) | 22(61.11%) | |
| Mean ± Sd | 14.11±10.95 | 16.1±10.67 | 20.38±14.6 | 0.08 |
| | 14.11±10.95 | | 20.38±14.6 | 0.04 |
| | | 16.1±10.67 | 20.38±14.6 | 0.15 |
| | 15.13±10.78 | | 20.38±14.6 | 0.03 |
| Time To Reach Birth Weight | Group A (37) | Group B (40) | Group C (36) | |
| 1-7 | 11(29.73%) | 10(25%) | 4(11.11%) | |
| 8-14 | 17(45.95%) | 19(47.5%) | 15(41.67%) | 0.15 |
| >14 | 9(24.32%) | 11(27.5%) | 17(47.22%) | |
| Mean±Sd | 11.11±5.42 | 11.73±5.29 | 15.36±8.02 | 0.01 |
| | 11.11±5.42 | | 15.36±8.02 | 0.01 |
| | | 11.73±5.29 | 15.36±8.02 | 0.02 |
| | 11.43±5.32 | | 15.36±8.02 | 0.003 |
| Weight Gain (Gm/Kg/Day) At 1 Month Of Age | Group A (32) | Group B (35) | Group C (30) | |
| 1.0-4.9 | 4(12.5%) | 9(25.71%) | 12(40%) | |
| 5.0-9.9 | 10(31.25%) | 9(25.71%) | 11(36.67%) | |
| 10-14.9 | 17(53.13%) | 12(34.28%) | 7(23.33%) | 0.02 |
| ≥15 | 1(3.13%) | 5(14.28%) | 0(0.00%) | |
| Mean±Sd | 9.65±3.72 | 8.98±4.49 | 6.58±3.86 | 0.01 |
| | 9.65±3.72 | | 6.58±3.86 | 0.002 |
| | | 8.98±4.49 | 6.58±3.86 | 0.03 |
| | 9.3±4.12 | | 6.58±3.86 | 0.003 |

## Discussion

In this open- labeled RCT, we evaluated the effect of probiotic Lactobacillus rhamnosus GG in low and high doses in preterm neonates <35 weeks on preventing NEC, LOS and various other morbidities.

In our study, no significant difference was observed in the reduction of NEC, LOS and mortality rate following the use of probiotic Lactobacillus rhamnosus GG in low dose as well as high doses. Out of 123 neonates, NEC developed in 2(4.88%) neonates in Group A (one had stage 1a and one had stage 2a NEC), 1(2.38%) neonate in Group B developed stage 2a NEC but none was reported in group C(p value 0.37). There was no statistically significant difference in the incidence of NEC between groups A and C (p value 0.16), between groups B and C (p value 0.33) and between groups A+B combined and group C (p value 0.23). In Group A, 5 newborns (12.2%) developed LOS (3 culture proven LOS, 1 suspected LOS & 1 clinically LOS). In Group B, 3 newborns

(7.14%) developed LOS (2 suspected LOS and 1 clinical LOS). In Group C, 3 newborns (7.5%) developed LOS (2 suspected LOS & 1 clinical LOS). There was no statistically significant difference between the three groups in regards occurrence of LOS (p value 0.7).There was no significant difference in immediate outcomes between 3 groups (p value 0.55).

We found two RCTs and three observational studies analogous to our study wherein Lactobacillus rhamnosus was used as a single strain. Similar to our findings, both RCTs did not show any significant reduction in NEC, LOS and mortality rate . In an RCT conducted by Dani et al in Italy (2002); the use of L. GG in 6 billion CFUs daily dose in preterm neonates < 33 weeks or < 1500 grams did not decrease the incidence of definite NEC (RR 0.49 (0.15, 1.61)), LOS (RR 1.15 (0.54, 2.44)) and mortality (RR 0.2 (0.01, 4.08))(9). Similarly, in a study by Manzoni et al (2006) (Italy) with use of Lactobacillus rhamnosus in 6 billion CFUs dose, there was no significant reduction in the risk of NEC (RR 0.15 (0.01 – 2.81, p 0.2), sepsis (RR 0.93 (0.54 – 1.59, p 0.78), mortality (RR 0.88 (0.29-2.64, p 0.81) and length of hospital stay (RR -5.0 (-17.73 – 7.73, p 0.44)) (10). In an retrospective observational study by Luoto et al.(Finland, 2010) L. GG in 6 billion CFUs daily dose had no significant effect on NEC stage 2 or 3 (RR 1.42 (0.86, 2.34))(11). In a retrospective observational cohort study by Kane et al. (USA) on over historical 2 different study periods in VLBW neonates with variable doses of L. GG between 2.5 to 5 CFU / day did not find reduction in NEC(12). The beneficial effect was only documented in an observational study by Bonsante (France, 2013) on neonates with > 24 weeks to < 31 weeks maturity, where in Lactobacillus rhamnosus was given in low doses of 0.4 billion CFU; There was a significant drop in severe NEC stage 2 – 3(RR 0.21 (0.08, 0.59)), in LOS (RR 0.64 (0.46, 0.9)) and mortality (RR 0.48 (0.22, 1.01))(13). Though this study was conducted on many extreme and very preterm neonates (a total of 1130 samples), it was retrospective and observational in nature between the historical cohort and probiotic cohort in 2 different study periods.

We found several other RCTs where in Lactobacillus rhamnosus GG was given in conjunction with other probiotics. Out of 7 RCTs, only the study conducted by Manzoni et al on VLBW neonates showed significant positive effect(16-21). They observed beneficial effects both in the bovine lactoferrin along with L. GG (6 billion CFUs daily) group and in the lactoferrin group as opposed to the control group(22).

Study which showed significant effect was of, who found beneficial effects in both

In a strain - specific network meta-analysis performed by Chris et al that covered RCTs till September 2017 (a total of 51 RCTs), L. rhamnosus GG (RR 0.24 (0.064, 0.67)) was ranked third in reduction of NEC grade 2 or 3 after B.lactis Bb 12 / B94 & L. Reuteri. However , there was no statistically significant reduction in LOS (RR 0.80 (0.47, 1.3)) and in mortality (RR 0.89 (0.32, 2.3)) with its use in isolation. The Combination of B. longum 35624 and L. rhamnosus GG ranked seventh in the reduction of NEC, the combination of B. longum, L. helveticus, L. rhamnosus, S. boulardii ranked second in the reduction of LOS and the combination of B. infantis, L. acidophillus, L. casei, L. plantarum, L. rhamnosus and S. thermophilus ranked third is the reduction of mortality.[7] A more recent network meta-analysis by Beghetti et al(2021) (covering trials till Jan 2020, consisting of 51 RCT trials) did not show a significant effect of L. rhamnosus GG in NEC prevention (RR 0.58 (0.23 – 1.37)) (8).

In our study, the beneficial effects of L. GG were observed as secondary outcome measures. The use of Lactobacillus rhamnosus GG particularly in higher doses was significantly associated with diminished incidence of hyperbilirubinemia. Further analysis of the presence of various risk factors leading to hyperbilirubinemia among the three groups, did not yield reveal any significant difference. It means that Lactobaccilus rhamnosus GG is probably helpful in reducing the incidence of hyperbilirubinemia. We did not find any studies evaluating the effect of Lactobacillus rhamnosus GG on neonatal hyperbilirubinemia.

Moreover , we found significant differences in time to reach birth weight in L. rhamnosus GG groups compared to the controls (p - value 0.003). Similarly, weight gain at 1 month of age was significantly better in groups A and B (L. GG) than group C (control) (p value 0.003). in the Literature we found discrete results with some studies reporting positive or negative outcomes. In a retrospective cohort study conducted by Meyer et al, days to regain birth weight was significantly dropped with the use of lactoferrin with L. GG (p value <0.001) (23). Deng et al conducted a retrospective study (L. rhamnosus GG with B. infantis in 113 neonates (Control) vs. 108 neonates(Study) with birth weight ≤ 1250 grams and/or ≤28 weeks), with the results suggesting

that the odds of EUGR was significantly lower in groups receiving probiotics (OR:0.3, 95% CI 0.138 to 0.611, P < 0.05)(24). In a meta-analysis done by Jung et al(covering a total of 15 RCTs), probiotics had no significant effect on weight gain (mean difference -0.29 (-1.16, 0.58), p 0.51) (3).

The Duration of hospital stay among discharged neonates was significantly lower in L. rhamnosus GG group than in the control group (p value 0.03), which may be attributed to possibility of recruiting neonates with higher birth weight, earlier reaching of full feeds and higher weight gain in the probiotics group.

Similar to other studies, we did not find any side effects for Lactobacillus rhamnosus GG in the study (4,19-22).

***Strengths & Limitations***

The main strength of the study is evaluating both low aand high doses of LGG in a single study setting apart from its good study design and adequate follow up .

The Major limitation of this study is its relatively small sample size, in particularly extremely and very preterm neonates in whom the overall risk of occurrence of NEC & LOS is higher. Post hoc power analysis of the study was 14% for NEC and 5% for LOS. Another limitation is that blinding was not ensured in our study. Also ,regarding one of the secondary outcomes i.e.– assessment of weight gain at 1 month of age, we were not able to do follow up of 6 neonates in group A, 5 in group B and 6 in group C.

## Conclusion

In conclusion, probiotic Lactobacillus rhamnosus GG in isolation as a single strain both in high and low doses has no significant effect on reducing the incidence of NEC, late onset sepsis and final outcomes. Lactobacillus rhamnosus GG is probably helpful in significant reduction in incidence of neonatal hyperbilirubinemia, in particularly in higher dosages. With use of lactobacillus rhamnosus GG can significantly decrease the time to reach birth weight and weight gain at one month of age, which may explain the shortened the duration of hospital stay. Given the smaller sample size of our study , it is suggested to perform a study with a larger sample size and a well blinded design before drawing definitive conclusion.

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## Conflicts of interest

None.

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