ARTICLE

Effects of prophylactic probiotics supplementation on infants born very preterm or very low birth weight

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OBJECTIVE: To evaluate the effects of guideline-driven prophylactic supplementation of a multi-strain neonatal intensive care unit-specific probiotic product on infants born very preterm (VP) or very low birth weight (VLBW).
STUDY DESIGN: A prospective cohort of 125 infants born in one year after implementation who received probiotics were compared to a retrospective cohort of eligible 126 VP or VLBW infants who did not receive probiotics. The primary outcome of interest was necrotizing enterocolitis (NEC).
RESULT: The incidence of NEC decreased from 6.3 to 1.6%. After adjusting for multiple variables, there were no significant differences in primary or other outcomes of interest; odds ratio (95% confidence interval) NEC 0.27 (0.05–1.33), death 0.76 (0.26–2.21) and late-onset sepsis 0.54 (0.18–1.63). No adverse effects related to probiotics supplementation were observed.
CONCLUSION: Although nonsignificant, prophylactic probiotics supplementation in infants born VP or VLBW was associated with reduction of NEC.

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INTRODUCTION

Necrotizing enterocolitis (NEC) remains a major cause of mortality and morbidity in very preterm (VP) infants [1, 2]. Although the etiopathogenesis is likely multifactorial, inappropriate initial microbial colonization in preterm infants appears to be the most important risk factor for NEC [3]. The immature gut with altered host defense may stimulate an excessive inflammatory response to this dysbiosis, eventually triggering cell injury [4]. The intestinal flora of patients with NEC has a predominance of proteobacteria and significant reduction in diversity of species [5]. Pneumatosis, the pathognomonic sign of NEC is due to abnormal bacterial fermentation.

Probiotics are live microorganisms, when administered in adequate amounts, confer a health benefit on the host [6]. Probiotics protect the immature gut by promoting more diverse intestinal flora, which competitively exclude certain pathogenic bacteria [7]. Therefore, optimizing the preterm gut microbiome with probiotics is a favorable strategy to prevent NEC [8]. A recent systematic review of randomized controlled trials (RCTs) for preventing NEC in preterm infants showed decreased risk of NEC by probiotics with moderate certainty of evidence [9].

Multiple previous meta-analyses have reported that enteral supplementation with probiotics compared to no supplementation or placebo, reduces the risk of NEC, sepsis and mortality [10, 11]. Although numerous trials published and a large number of patients studied, the heterogeneity in study methods and uncertainty regarding efficacy of different probiotic preparations make it difficult to generalize the results. Moreover, given the declining incidence of NEC, it may be challenging to conduct large RCTs adequately powered to detect clinically important reductions [12]. Currently in the United States, the routine use of probiotics in the neonatal intensive care units (NICUs) is limited [13, 14].

The objective of this pre- and post-implementation cohort study was to evaluate the effects of routine prophylactic supplementation of a multi-strain NICU-specific probiotic product in infants born very preterm (<32 weeks of gestation at birth) or very low birth weight (<1500 grams, VLBW) in our practice located in North Texas. We hypothesized that a guideline-driven supplementation of probiotics would be safe, feasible and associated with a decrease in incidence of NEC from the baseline of 6.3% (one year prior to implementation) to 3.3% (one year after implementation) based on estimates derived using data from meta-analysis by Sawh et al. [10, 15].

METHODS

Patients and setting

Our neonatology practice cares for approximately 150 infants born VP or VLBW every year. Prior to implementation, probiotics were not utilized for any reason. A guideline was prepared to prophylactically supplement infants born VP or VLBW with Similac® Probiotic Triblend (Abbott, Abbott Park, Illinois; manufactured by Chr. Hansen, HØrsholm, Denmark) from July 1, 2020 in a level IV NICU and from October 1, 2020 in a level III NICU.

One single-use packet (0.5 g powder) of Similac® Probiotic Triblend has a combination of one billion colony forming units (CFU) of *Bifidobacterium*

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lactis (BB-12®), Bifidobacterium infantis (BB-02™), and *Streptococcus thermophilis (TH-4®)*.

Guideline

The probiotic packet was mixed in 3 ml of sterile water in a dedicated place by the nurse. The first dose of probiotic was given within 48 h of birth, after first feed of mother's colostrum (or donor breast milk if colostrum was not available). It was administered through the gavage tube replacing a feed equivalent to the feeding volume if the infant was receiving <3 ml of feed. If the feed was ≥3 ml, the 3 ml probiotic was given in addition to the feed. The probiotic was followed by a flush of 0.5 ml of sterile water. Subsequently, the probiotic was dosed once a day until the infant reached 35 weeks post menstrual age (PMA) or discharge (whichever was earlier). Contraindications to the administration of probiotics included any breach in the gut integrity from conditions such as spontaneous intestinal perforation (SIP), NEC Bell's stage ≥2.

Study design

This prospective cohort study with a retrospective cohort comparison was conducted after approval by the local institutional review board. Exclusion criteria included death or discharge prior to the first dose of probiotics, death within 48 h after birth or complex congenital or chromosomal anomalies. The prospective and retrospective data were extracted from maternal and neonatal electronic medical records. The prospective study period was 1-year post-implementation, from July 1, 2020 to June 30, 2021 for the level IV NICU and October 1, 2020 to September 30, 2021 for the level III NICU. The study period for the retrospective cohort was also 1-year pre-implementation, from July 1, 2019 to June 30, 2020 for the level IV NICU and October 1, 2019 to September 30, 2020 for the level III NICU.

Data collection

Collected data included maternal demographics, obstetric complications and other labor and delivery variables. Neonatal data such as gestational age, birth weight, gender, Apgar scores, incidence of small for gestational age (SGA, birth weight <10th percentile) and hypothermia (first temperature in the NICU < 96.8°F) were recorded. Nutrition data included the age of first feed, age when probiotics were started, days on parenteral nutrition, central line days and days to full feeds (140 mL/kg/day). Growth velocity was calculated from daily weights by an exponential model and expressed as g/kg/day [16]. Incidence of cholestasis (direct bilirubin >2 mg/dl), postnatal growth failure (discharge weight <10th percentile) and change in z-score between birth and discharge weights were noted.

Outcomes

The primary outcome of interest was NEC. The other outcomes of interest were death and late-onset sepsis (LOS). NEC was described based on clinical and radiographic criteria [17]. Infants must have at least one of the following clinical signs: bilious gastric aspirate or emesis, abdominal distension or occult/gross blood in stool (no fissure) and at least one of the following radiographic findings: pneumatosis intestinalis, portal venous gas, or pneumoperitoneum. NEC needing surgery (surgical NEC) was specifically differentiated from SIP. SIP was defined as focal gastrointestinal perforation diagnosed by visual inspection at the time of surgery or postmortem exam with remainder of the bowel normal [17]. LOS was diagnosed when pathogen was isolated from blood or cerebrospinal fluid >72 h after birth, or a postmortem culture of organ tissue grew a pathogen with concomitant histology of infection. In addition, the number of LOS evaluations >72 h after birth (at least a blood culture obtained plus initiation of antibiotics) were recorded. Other major neonatal outcomes included bronchopulmonary dysplasia, retinopathy of prematurity, and intraventricular hemorrhage. Diagnosis of bronchopulmonary dysplasia was made at 36 weeks post menstrual age if there was any oxygen requirement. In addition, if the infant was on continuous positive airway pressure or ventilator at 36 weeks post menstrual age, it was labeled as severe bronchopulmonary dysplasia [18]. Retinopathy of prematurity needing any treatment was also documented [19]. Intraventricular hemorrhage was graded 1–4 based on the criteria developed by Papile et al. who defined grades 3 and 4 as severe intraventricular hemorrhage [20].

Statistical analyses

In this cohort study a study time period of one year each was chosen for pre- and post-implementation with a goal of 125 VP or VLBW infants in each group. Demographic and outcome variables were compared between the probiotics and no probiotics groups utilizing a 2-tailed Student's t test or Mann-Whitney U test for continuous variables and Pearson chi-square test or Fisher's exact test for categorical variables. Logistic regression was performed on primary outcome and other outcomes of interest after adjusting for variables such as gestational age, birth weight, gender, SGA and delayed cord clamping (DCC). These results were reported as odds ratios (ORs) with 95% confidence intervals (CIs). A subgroup analysis was also performed on infants born extremely low birth weight (<1000 grams, ELBW). IBM SPSS version 28 (Armonk, NY) was used to analyze data. A probability value <0.05 was considered to be the threshold of statistical significance.

RESULTS

During the prospective study period, out of 131 VP and/or VLBW infants admitted to the NICU, 6 were excluded (3 died before probiotics were started or within 48 h after birth and 3 were born with complex congenital anomalies). The remaining 125 VP and/or VLBW infants who received probiotics (Probiotics group) were compared to a retrospective cohort of 126 infants who survived beyond 48 h and qualified to receive probiotics, but did not as they were born prior to implementation of routine supplementation (No probiotics group).

No significant differences emerged for any maternal characteristics (Table 1). The median gestational age at birth was 29 weeks and median birth weight was 1200 grams in both the groups. Although there was no significant difference in the incidence of LOS, the median evaluations for suspected LOS were significantly lower in the Probiotics group compared to No probiotics group. The incidence of NEC decreased from 6.3% in the No probiotics group to 1.6% in the Probiotics group. The other characteristics and outcomes, including the incidence of death were similar between both groups (Table 2). The first feed was given significantly earlier after birth in the Probiotics group compared to No probiotics group (15 vs. 17 h of life). The median age of starting probiotics was 33 h of life and were given until 35 weeks PMA. The median number of probiotic doses given were 35. The growth velocity was significantly higher in the Probiotics group compared to No probiotics group (14 vs. 13 g/kg/day). Similarly, median change in z-score between the birth and discharge weights decreased significantly in the Probiotics group compared to No probiotics group (−0.57 vs. −0.80) (Table 3).

Multiple logistic regression after adjusting for birth weight, gestational age, gender, SGA and DCC revealed no differences in the primary outcome or other outcomes of interest (NEC, death, NEC or death and LOS) for VP or VLBW infants (Table 4). In the subgroup analysis for only ELBW infants, there were no cases of NEC in the Probiotics group compared to the incidence of 14.3% in the No probiotics group. There were no significant differences in the other outcomes of interest between both the groups (Table 5).

DISCUSSION

Recent meta-analyses of randomized and non-randomized studies, including 56 RCTs and 30 high quality observational trials respectively comparing probiotics to placebo or no probiotics showed a reduction in NEC with consistent effect size [11, 21]. However, this evidence remains a low to moderate certainty due to heterogeneity in trial designs, small sample sizes in the individual trials, and usage of different probiotic preparations. The RCTs addressing NEC are also confounded with variations in the definition and creating cohorts confounded by SIP [22]. This is quoted as one of the reasons for uncertainty regarding the benefits of probiotics in reducing NEC. Thus, it is very important to improve criteria to define NEC in a consistent way for quality improvement and research purposes [23, 24]. However, it may be challenging to conduct large adequately powered trials to detect significant reduction given the declining incidence of NEC [12].

Table 1. Maternal characteristics.

Characteristic	Retrospective [No probiotics] (*N* = 126) *n* (%)	Prospective [Probiotics] (*N* = 125) *n* (%)	*P* value
White	49 (38.9)	45 (36)	0.62
Hypertensive disorders of pregnancy	42 (33.3)	43 (34.4)	0.90
Diabetes	18 (14.3)	23 (18.4)	0.40
Multiple gestation	34 (27)	38 (30.4)	0.60
Intrauterine growth restriction	24 (19)	24 (19.2)	>0.99
Oligohydramnios	8 (6.3)	12 (9.6)	0.34
Antenatal steroids	112 (88.9)	112 (89.6)	0.90
Magnesium	102 (81)	107 (85.6)	0.32
Group B Streptococcus positive	22 (17.5)	13 (10.4)	0.11
Chorioamnionitis	6 (4.8)	8 (6.4)	0.60
Histological chorioamnionitis	25/82 (30.5)	33/104 (31.7)	0.80
Intrapartum antibiotic prophylaxis	61 (48.4)	56 (44.8)	0.60
Preterm labor	67 (53.2)	63 (50.4)	0.70
Rupture of membranes	48 (38.1)	53 (42.4)	0.50
Cesarean section	88 (69.8)	84 (67.2)	0.70
Placental abruption	10 (7.9)	7 (5.6)	0.50

According to a phone survey performed in 2015, only 8.8% of the participating NICUs in the US were using probiotics routinely for the VLBW infants. When explored, there was no evidence for the safety or efficacy for most of the probiotic products used at that time [13]. Only one product was reported to have protective effect against NEC [25]. This probiotic combination of *B. infantis, S. thermophilus* and *B. lactis*, registered with the German Collection of Microorganisms and Cell Cultures as BB-02 96579, Th-4 15957, BB-12 15954 respectively (ABC Dophilus Probiotic Powder for infants, Solgar, Leonia, New Jersey) has been tested in a well-designed RCT (ProPrems study) where 1099 very preterm infants from Australia and New Zealand were randomized to either probiotics or placebo. Although no significant reduction was noted in LOS or mortality, this probiotic combination reduced NEC Bell's stage ≥2 with a number needed to treat of 43 [26]. It appeared to be safe and did not adversely affect neurodevelopment or behavior in early childhood [27]. Therefore, this combination is conditionally recommended by the panel of experts representing European Society for Pediatric Gastroenterology Hepatology and Nutrition Committee to reduce NEC Bell's stage ≥2 if all safety conditions are met [28].

A systematic review and network meta-analysis of preterm infants which included 63 trials has shown with high-certainty evidence that compared to placebo or single-strain or other multi-strain probiotic formulations, combinations of one or more *Lactobacillus* species and one or more *Bifidobacterium* species are superior for the reduction of mortality and NEC Bell's stage ≥2 with no difference in the incidence of harm [29]. Another network meta-analysis of preterm infants reported that only a minority of studied strains or combinations had efficacy in reducing mortality or morbidities [30]. The published systematic reviews do recognize that there is not enough information at the strain level in the included trials. Moreover, the validity and viability of the live microbes is rarely reported. In the current study, routine supplementation with multi-strain probiotic blend consisting of *Bifidobacterium lactis (BB-12®), Bifidobacterium infantis (BB-02™)* and *Streptococcus thermophilis (TH-4®)* to the infants born VP or VLBW for one year was associated with the reduction of NEC from the baseline of 6.3% to 1.6%. This was better than the aimed risk reduction of 47% (6.3 to 3.3%) based on the published estimates derived from meta-analysis by Sawh et al. (RR of NEC 0.53, 95% CI 0.42–0.66) [10, 15]. Although this reduction of 75% in the current study was not statistically significant, it is clinically relevant. Nonsignificant reductions were also noted with the incidence of LOS decreasing from 10.3% to 5.3% and mortality from 9.5% to 6.4% after introduction of routine probiotics supplementation. For the Similac® Probiotic Triblend utilized in the current study, the manufacturer Chr. Hansen discloses that all three strains were isolated from either their collection of dairy cultures or in-licensed by trusted partners. They underwent genomic sequencing which provide a comprehensive insight of the characteristics and functionality of the strains [31]. These strains are reported to carry no transferable antibiotic-resistant genes. Deoxyribonucleic acid fingerprinting is performed to confirm their validity [32].

Presently, the practice guidelines from major societies affirm the insufficient evidence regarding safety and efficacy of probiotics in preventing NEC in ELBW infants [33, 34]. In a large meta-analysis, only 7 out of 56 RCTs included ELBW infants that showed no significant reduction in NEC, hodeath or LOS with probiotics [11]. However, the meta-analysis of observational trials showed a reduction in NEC Bell's stage ≥2 with probiotics in a subset of ELBW infants [21]. A cohort study utilizing a multicenter clinical database showed that even though the use increased over a period of two decades (1997–2016), only 4.6% of infants born between gestational age 23–29 weeks were treated with probiotics. In that study, probiotic use was associated with decreased odds of NEC and death [14]. In the current study, benefit was noted in the subset of ELBW infants with reduction in the incidence of NEC from 14.3% prior to 0% after routine implementation of probiotics. However, these results need to be interpreted with caution due to small sample-size.

The action of probiotics at the level of gut epithelium includes upregulation of cellular immunity and cytoprotective genes, enhanced barrier function, downregulation of pro-inflammatory pathways, prevention of apoptosis and competitive exclusion of pathogenic bacteria [7]. The exact mode of action is difficult to assess as it may be strain-dependent [35]. Preterm infants with suboptimal gut epithelial cell barrier and immune function are predisposed to pathogenic bacteria [36]. This in turn can trigger an inflammatory response including toll-like receptor (TLR) signaling. Expression of TLR4 may be critical in the pathogenesis of NEC [37]. Probiotics, by promoting normal commensal gut flora

Table 2. Neonatal characteristics and outcomes.

Characteristic	Retrospective [No probiotics] (*N* = 126) *n* (%)	Prospective [Probiotics] (*N* = 125) *n* (%)	*P* value
Inborn	111 (88.1)	115 (92)	0.30
Gestational age, weeks[a]	29 (26.9–30.7)	29.4 (27.3–30.7)	0.40
Birth weight, grams[a]	1190 (880–1513)	1230 (965–1404)	0.70
Small for gestational age	18 (14.3)	19 (15.2)	0.84
Female	61 (48.4)	48 (38.4)	0.11
Delayed cord clamping	78 (61.9)	92 (73.6)	0.05
Apgar scores, *n*[a]			
1-min	6 (3–7)	6 (4–8)	0.60
5-min	8 (7–9)	8 (7–9)	0.61
Intubation in the delivery room	39 (31)	36 (28.8)	0.90
Hypothermia on admission	8 (6.3)	5 (4)	0.40
Respiratory distress syndrome needing surfactant	64 (50.8)	53 (42.4)	0.20
Early onset sepsis	1 (0.8)	1 (0.8)	>0.99
Late-onset sepsis	13 (10.3)	7 (5.6)	0.20
Days on antibiotics, *n*[a]	2 (0–6)	2 (0–4)	0.20
Days on antibiotics after 72 h of age, *n*[a]	0 (0–3)	0 (0–1)	0.21
LOS evaluations, *n*[a]	0 (0–1)	0 (0–1)	0.03
Central line associated blood stream infection	2/109 (1.8)	2/103 (1.9)	0.70
Pneumonia	7 (5.6)	6 (4.8)	0.80
Death	12 (9.5)	8 (6.4)	0.40
Necrotizing enterocolitis	8 (6.3)	2 (1.6)	0.10
Surgical	4 (50)	2 (100)	0.50
Spontaneous intestinal perforation	5 (4)	3 (2.4)	0.72
NEC or SIP	12 (9.5)	5 (4)	0.10
Bronchopulmonary dysplasia	31/113 (27.4)	26/114 (22.8)	0.70
Severe	8 (25.8)	5 (19.2)	0.53
Retinopathy of prematurity	30/101 (29.7)	26/109 (23.8)	0.90
Surgical	1 (3.3)	4 (15.4)	0.20
Intracranial hemorrhage	35/124 (28.2)	21/122 (17.2)	0.08
Severe	10 (28.6)	8 (38.1)	0.50
Length of hospital stay, *n*[a]	67 (42–85)	61 (41–79)	0.40

[a]Median (interquartile range).

early in life, confers a protective effect against conditions associated with dysbiosis such as NEC and LOS [38]. These microbiota may also interact with immune system to aid in the establishment of normal digestive capacity, gut motility, ability to harvest nutrients and tolerance to food [39]. A RCT reported no effect on postnatal growth in preterm infants receiving Bifidobacterium supplements [40]. In the current study, there was a significant improvement in the growth velocity and significant decrease in the change of z-score between the birth and discharge weight in the group that received probiotics compared to the group that did not. In general, the effect on growth may vary depending on species and strains of microorganisms utilized [41].

Contamination of dietary supplement-grade probiotics products has been noted previously. Fatal gastrointestinal mucormycosis was reported in a preterm infant who received contaminated ABC Dophilus Powder [42]. Therefore, it is pivotal to choose products with elevated safety and quality standards and preferably single-use packets or vials. A recently published systematic review reported that probiotic sepsis is an uncommon event in preterm infants [43]. However, it is crucial that the departments of microbiology and pathology in the hospital are involved for regular surveillance. In the current study, there were no cases of sepsis or adverse events associated with probiotics. Probiotics administration was paused during the events when there was suspicion of compromise in infant's gut integrity. In such instances like SIP or NEC Bell's stage ≥2, there is a possibility of gut bacteria seeping into the blood stream. Probiotics were resumed when feeds were restarted. Routine anaerobic cultures were not performed when LOS was suspected. In general, the conventional biochemical methods utilized in the aerobic blood cultures inoculated for 5 days may help to identify the initial growth. If probiotic bacteremia is detected, genomic analysis can help in confirming the strains. This bacteremia is usually susceptible to the regular late-onset sepsis empiric antibiotics.

For the units choosing to introduce routine probiotics into practice, multidisciplinary approach with involvement of key stakeholders, consistent guideline, and methods to monitor outcomes are recommended. It is strongly suggested that other important NEC reduction strategies are in place such as

Table 3. Nutrition data.

Variable	Retrospective [No probiotics] (*N* = 126) *n* (%)	Prospective [Probiotics] (*N* = 125) *n* (%)	*P* value
Age feeds started, hours[a]	17 (9–28)	15 (7–24)	0.02
Feeds initiated with human milk	126 (100)	125 (100)	NA
Age probiotic started, hours[a]	NA	33 (17–48)	NA
Growth velocity, (g/kg/day)[a]	13 (11–14)	14 (12–15)	0.02
Days on TPN, *n*[a]	11 (7–15)	8 (6–11)	0.11
Days to full feeds, *n*[a]	11 (9–14)	9 (7–12)	0.20
Days of nil per os, *n*[a]	0 (0–1)	0 (0–0)	0.31
Central line days, *n*[a]	11 (6–16)	8 (8–13)	0.09
Cholestasis	7 (5.6)	7 (5.6)	0.22
Diaper rash	5 (4)	3 (2.4)	0.72
Probiotics stopped at PMA, weeks[a]	NA	35 (35–35)	NA
Probiotic doses, *n*[a]	NA	35 (3–83)	NA
Discharge on mother's milk	41/111 (36.9)	54/112 (48.2)	0.22
Postnatal growth failure	14/110 (12.7)	11/111 (9.9)	0.80
Change in z-score between birth and discharge weight, *n*[a]	−0.80 (−1.2 to −0.37)	−0.57 (−0.91 to −0.25)	0.03

TPN Total parenteral nutrition, *PMA* Post menstrual age.
[a]Median (interquartile range).

Table 4. Outcomes of interest for infants born very preterm or very low birth weight.

Variable	Prospective [Probiotics] (*N* = 125) *n* (%)	Retrospective [No Probiotics] (*N* = 126) *n* (%)	OR* (95% CI)	*P* value
Gestational age, weeks[a]	29.4 (27.3–30.7)	29 (26.9–30.7)		0.40
Birth weight, grams[a]	1230 (965–1404)	1190 (880–1513)		0.70
Small for gestational age	19 (15.2)	18 (14.3)		0.84
Female	48 (38.4)	61 (48.4)		0.11
Delayed cord clamping	92 (73.6)	78 (61.9)		0.05
Necrotizing enterocolitis	2 (1.6)	8 (6.3)	0.27 (0.05–1.33)	0.12
Death	8 (6.4)	12 (9.5)	0.76 (0.26–2.21)	0.62
NEC or death	9 (7.2)	16 (12.7)	0.61 (0.21–1.63)	0.34
Late-onset sepsis	7 (5.6)	13 (10.3)	0.54 (0.18–1.63)	0.28

*Adjusted for birth weight, gestational age, SGA, gender and DCC.
[a]Median (interquartile range).

optimization of human milk, standard feeding protocol, antibiotic stewardship and DCC. These initiatives have proven to help reduce NEC and may act in synergism with probiotics [15]. Administering human milk rich in oligosaccharides may improve colonization of the gut with the administered probiotic [44]. In the current study, 100% of the VP or VLBW infants were started on human milk (mother's milk or donor breast milk) early (within 24 h of life). Approximately 50% infants were discharged on mother's milk. A recently published meta-analysis suggests that combination of prebiotic and probiotic may have greater treatment efficacy than probiotic alone [45].

As a prospective cohort study with a historic cohort comparison, this study has several limitations. This study reports observational data one-year pre- and one-year post-implementation of routine probiotics supplementation. The enrollment was not based on sample-size calculation (to achieve statistical significance for a reduction of NEC from 6.3% to 3.3%, a sample-size of 1592 infants is needed). Moreover, a RCT may not have been feasible due to possible cross-colonization in the group not receiving probiotics. As a cohort study, the conclusions from this trial may be limited to associations. Also, there is a risk of confounding the results of the study by other practice changes. However, the other NEC reduction practices have been in place in our practice for many years. We did adjust for gestational age, birth weight, gender, SGA and DCC as these variables may influence the outcomes of interest. The radiologists reading the X-rays were not masked formally, but they were not aware of implementation of routine probiotics supplementation. A risk of industry bias cannot be ignored, even though this study did not receive any sponsorship and financial support. Despite these limitations, we feel it is important to share our observations, which are more likely to reflect the real-world clinical practice. In the current study, NEC was consistently defined based on clinical and radiographic criteria in both retrospective and prospective cohorts. In addition,

Table 5. Outcomes of interest for infants born extremely low birth weight.

Variable	Prospective Probiotics ($N = 34$) *n* (%)	Retrospective No Probiotics ($N = 42$) *n* (%)	OR* (95% CI)	*P* value
Gestational age, weeks[a]	25.4 (24.7–27.4)	26 (24.1–27.4)		>0.99
Birth weight, grams[a]	730 (576–890)	740 (556–893)		0.84
Small for gestational age	8 (23.5)	11 (26.2)		0.80
Female	15 (44.1)	21 (50)		0.61
Delayed cord clamping	23 (67.6)	18 (42.9)		0.03
Necrotizing enterocolitis	0 (0)	6 (14.3)	0.08 (0.004–1.53)[b]	0.10
Death	7 (20.6)	8 (19)	1.53 (0.38–5.72)	0.56
NEC or death	7 (20.6)	11 (26.2)	0.91 (0.24–3.41)	0.89
Late-onset sepsis	7 (20.6)	11 (26.2)	0.79 (0.23–2.64)	0.71

*Adjusted for birth weight, gestational age, SGA, gender and DCC.
[a]Median (interquartile range).
[b]Haldane-Anscombe correction.

we carefully differentiated SIP and NEC to avoid contamination of data. No other probiotics were used in the unit before or concurrently other than the specific implemented product. As there is limited data from the United States, our experience with a multi-strain probiotic product specifically available for NICU patients may add valuable information to the body of evidence on probiotics supplementation in premature infants. Although meta-analyses suggest benefit with the routine use of probiotics, they offer limited guidance in terms of which probiotics should be used, their dosage, which age group they provide greatest benefit for, and how long they should be given. We believe this observational study provides guidance and addresses some of the concerns that are impeding the widespread practice of routine probiotic supplementation in preterm infants.

In conclusion, routine prophylactic supplementation of a NICU-specific multi-strain probiotic in infants born VP or VLBW was associated with reduction of NEC that is consistent with the literature-reported effect size with no adverse effects such as probiotic sepsis. This study adds to the evidence supporting the routine supplementation of probiotics in infants born VP or VLBW. Further studies need to focus on the optimal length of therapy, additional NICU populations that may benefit, and other effective probiotic and symbiotic combinations.

DATA AVAILABILITY

The de-identified dataset generated from the study is available on reasonable request and upon approval by the Institutional Review Board of Baylor Scott & White Health.

REFERENCES

1. Jacob J, Kamitsuka M, Clark RH, Kelleher AS, Spitzer AR. Etiologies of NICU deaths. Pediatrics. 2015;135:e59–65.
2. Bell EF, Hintz SR, Hansen NI, Bann CM, Wyckoff MH, DeMauro SB, et al. Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Mortality, In-Hospital Morbidity, Care Practices, and 2-Year Outcomes for Extremely Preterm Infants in the US, 2013-2018. JAMA. 2022;327:248–63.
3. Claud EC, Walker WA. Hypothesis: inappropriate colonization of the premature intestine can cause neonatal necrotizing enterocolitis. FASEB J. 2001;15:1398–403.
4. Hunter CJ, Upperman JS, Ford HR, Camerini V. Understanding the susceptibility of the premature infant to necrotizing enterocolitis (NEC). Pediatr Res. 2008;63:117–23.
5. Wang Y, Hoenig JD, Malin KJ, Qamar S, Petrof EO, Sun J, et al. 16S rRNA gene-based analysis of fecal microbiota from preterm infants with and without necrotizing enterocolitis. ISME J. 2009;3:944–54.
6. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11:506–14.
7. Halloran K, Underwood MA. Probiotic mechanisms of action. Early Hum Dev. 2019;135:58–65.
8. Patel RM, Underwood MA. Probiotics and necrotizing enterocolitis. Semin Pediatr Surg. 2018;27:39–46.
9. Xiong T, Maheshwari A, Neu J, Ei-Saie A, Pammi M. An Overview of Systematic Reviews of Randomized-Controlled Trials for Preventing Necrotizing Enterocolitis in Preterm Infants. Neonatology. 2020;117:46–56.
10. Sawh SC, Deshpande S, Jansen S, Reynaert CJ, Jones PM. Prevention of necrotizing enterocolitis with probiotics: a systematic review and meta-analysis. PeerJ. 2016;4:e2429.
11. Sharif S, Meader N, Oddie SJ, Rojas-Reyes MX, McGuire W. Probiotics to prevent necrotising enterocolitis in very preterm or very low birth weight infants. Cochrane Database Syst Rev. 2020;10:CD005496.
12. Razak A, Patel RM, Gautham KS. Use of Probiotics to Prevent Necrotizing Enterocolitis: Evidence to Clinical Practice. JAMA Pediatr. 2021;175:773–4.
13. Viswanathan S, Lau C, Akbari H, Hoyen C, Walsh MC. Survey and evidence-based review of probiotics used in very low birth weight preterm infants within the United States. J Perinatol. 2016;36:1106–11.
14. Gray KD, Messina JA, Cortina C, Owens T, Fowler M, Foster M, et al. Probiotic Use and Safety in the Neonatal Intensive Care Unit: A Matched Cohort Study. J Pediatr. 2020;222:59–64.e1.
15. Barbian ME, Buckle R, Denning PW, Patel RM. To start or not: Factors to consider when implementing routine probiotic use in the NICU. Early Hum Dev. 2019;135:66–71.
16. Patel AL, Engstrom JL, Meier PP, Kimura RE. Accuracy of methods for calculating postnatal growth velocity for extremely low birth weight infants. Pediatrics. 2005;116:1466–73.
17. Vermont Oxford Network. 2021 Manual of Operations, Part 2, Release 25.0. 2021. https://vtoxford.zendesk.com/hc/en-us/articles/360056768093-2021-Manual-of-Operations-Part-2-Release-25-0-PDF.
18. Ehrenkranz RA, Walsh MC, Vohr BR, Jobe AH, Wright LL, Fanaroff AA, et al. National Institutes of Child Health and Human Development Neonatal Research Network. Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. Pediatrics. 2005;116:1353–60.
19. Chiang MF, Quinn GE, Fielder AR, Ostmo SR, Paul Chan RV, Berrocal A, et al. International Classification of Retinopathy of Prematurity, Third Edition. Ophthalmology. 2021;128:e51–68.
20. Papile L-A, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1500 gm. J Pediatr. 1978;92:529–34.
21. Deshmukh M, Patole S. Prophylactic Probiotic Supplementation for Preterm Neonates-A Systematic Review and Meta-Analysis of Nonrandomized Studies. Adv Nutr. 2021;12:1411–23.
22. Swanson JR, Hair A, Clark RH, Gordon PV. Spontaneous intestinal perforation (SIP) will soon become the most common form of surgical bowel disease in the extremely low birth weight (ELBW) infant. J Perinatol. 2022;42:423–9.

23. Neu J. Necrotizing Enterocolitis: The Future. Neonatology. 2020;117:240–4.
24. Patel RM, Ferguson J, McElroy SJ, Khashu M, Caplan MS. Defining necrotizing enterocolitis: current difficulties and future opportunities. Pediatr Res. 2020;88:10–5.
25. Bin-Nun A, Bromiker R, Wilschanski M, Kaplan M, Rudensky B, Caplan M, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. J Pediatr. 2005;147:192–6.
26. Jacobs SE, Tobin JM, Opie GF, Donath S, Tabrizi SN, Pirotta M, et al. ProPrems Study Group. Probiotic effects on late-onset sepsis in very preterm infants: a randomized controlled trial. Pediatrics. 2013;132:1055–62.
27. Jacobs SE, Hickey L, Donath S, Opie GF, Anderson PJ, Garland SM, et al. Pro-PremsStudy Groups. Probiotics, prematurity and neurodevelopment: follow-up of a randomised trial. BMJ Paediatr Open. 2017;1:e000176.
28. van den Akker CHP, van Goudoever JB, Shamir R, Domellöf M, Embleton ND, Hojsak I, et al. Probiotics and Preterm Infants: A Position Paper by the European Society for Paediatric Gastroenterology Hepatology and Nutrition Committee on Nutrition and the European Society for Paediatric Gastroenterology Hepatology and Nutrition Working Group for Probiotics and Prebiotics. J Pediatr Gastroenterol Nutr. 2020;70:664–80.
29. Morgan RL, Preidis GA, Kashyap PC, Weizman AV, Sadeghirad B, McMaster Probiotic, Prebiotic, and Synbiotic Work Group. Probiotics Reduce Mortality and Morbidity in Preterm, Low-Birth-Weight Infants: A Systematic Review and Network Meta-analysis of Randomized Trials. Gastroenterology. 2020;159:467–80.
30. van den Akker CHP, van Goudoever JB, Szajewska H, Embleton ND, Hojsak I, Reid D, et al. ESPGHAN Working Group for Probiotics, Prebiotics & Committee on Nutrition. Probiotics for Preterm Infants: A Strain-Specific Systematic Review and Network Meta-analysis. J Pediatr Gastroenterol Nutr. 2018;67:103–22.
31. Jungersen M, Wind A, Johansen E, Christensen JE, Stuer-Lauridsen B, Eskesen D. The Science behind the Probiotic Strain Bifidobacterium animalis subsp. lactis BB-12(®). Microorganisms. 2014;2:92–110.
32. Data on file. Abbott Nutrition. 2020. https://static.abbottnutrition.com/cms-prod/abbottnutrition-2016.com/img/Similac-Probiotic-Tri-Blend.pdf.
33. Poindexter B. COMMITTEE ON FETUS AND NEWBORN. Use of Probiotics in Preterm Infants. Pediatrics. 2021;147:e2021051485.
34. Marchand V. Using probiotics in the paediatric population. Paediatr Child Health. 2012;17:575–6.
35. Martin R, Nauta AJ, Ben Amor K, Knippels LM, Knol J, Garssen J. Early life: gut microbiota and immune development in infancy. Benef Microbes. 2010;1:367–82.
36. Torrazza RM, Neu J. The altered gut microbiome and necrotizing enterocolitis. Clin Perinatol. 2013;40:93–108.
37. Hackam DJ, Sodhi CP. Toll-Like Receptor-Mediated Intestinal Inflammatory Imbalance in the Pathogenesis of Necrotizing Enterocolitis. Cell Mol Gastroenterol Hepatol. 2018;6:229–38.e1.
38. Gritz EC, Bhandari V. The human neonatal gut microbiome: a brief review. Front Pediatr. 2015;3:17.
39. Groer MW, Luciano AA, Dishaw LJ, Ashmeade TL, Miller E, Gilbert JA. Development of the preterm infant gut microbiome: a research priority. Microbiome. 2014;2:38.
40. Hays S, Jacquot A, Gauthier H, Kempf C, Beissel A, Pidoux O, et al. Probiotics and growth in preterm infants: A randomized controlled trial, PREMAPRO study. Clin Nutr. 2016;35:802–11.
41. Million M, Angelakis E, Paul M, Armougom F, Leibovici L, Raoult D. Comparative meta-analysis of the effect of lactobacillus species on weight gain in humans and animals. Micro Pathog. 2012;53:100e8.
42. Vallabhaneni S, Walker TA, Lockhart SR, Ng D, Chiller T, Melchreit R, et al. Centers for Disease Control and Prevention (CDC). Notes from the field: Fatal gastrointestinal mucormycosis in a premature infant associated with a contaminated dietary supplement—Connecticut, 2014. MMWR Morb Mortal Wkly Rep. 2015;64:155–6.
43. Kulkarni T, Majarikar S, Deshmukh M, Ananthan A, Balasubramanian H, Keil A, et al. Probiotic sepsis in preterm neonates-a systematic review. Eur J Pediatr. 2022;181:2249–62.
44. Jena PK, Sheng L, Nagar N, Wu C, Barile D, Mills DA, et al. The effect of synbiotics *Bifidobacterium infantis* and milk oligosaccharides on shaping gut microbiota community structure and NASH treatment. Data Brief. 2018;19:1025–9.
45. Chi C, Li C, Buys N, Wang W, Yin C, Sun J. Effects of Probiotics in Preterm Infants: A Network Meta-analysis. Pediatrics. 2021;147:e20200706.

AUTHOR CONTRIBUTIONS

AC contributed to conceptualization/design, methodology, data curation, formal analysis and drafted the paper. HH and RH contributed to the methodology, data curation, formal analysis and drafted the paper. AR and JR contributed to the methodology, data curation and reviewed the manuscript. SD contributed to the methodology, formal analysis and reviewed the paper. MS contributed to conceptualization/design, methodology and reviewed the paper.

COMPETING INTERESTS

AC has received speaking honoraria from Abbott nutrition. The authors declare no competing interests.

ADDITIONAL INFORMATION

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