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QUALITY IMPROVEMENT ARTICLE

Implementation of a probiotic protocol to reduce rates of necrotizing enterocolitis

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Abstract

Objective To utilize a probiotic protocol to achieve a 50% reduction in rates of necrotizing enterocolitis (NEC) ≥ Bell Stage 2 within 2 years of protocol implementation.

Study design Literature review guided probiotic selection and protocol design. A driver diagram identified key drivers to achieve our aim. A U chart followed monthly NEC ≥ Bell Stage 2 per 100 patient days and per monthly admissions. The process measure was protocol compliance and the balancing measure was probiotic sepsis.

Results NEC ≥ Bell Stage 2 decreased from 0.14 to 0.04 per 100 patient days in infants < 33 weeks gestation or <1500 g, or a yearly rate of 7–2%. Protocol compliance was 98% and there were no cases of probiotic sepsis.

Conclusion Implementation of a probiotic protocol was associated with a decrease in rates of NEC.

Introduction

Approximately 7% of infants < 1500 g develop necrotizing enterocolitis (NEC) [1]. Mortality rates are 20–30%, with survivors at increased risk for neurodevelopmental delay, short bowel syndrome, and poor growth [1, 2]. The pathophysiology of NEC is multifactorial and includes immature innate immunity, formula feeding, intestinal hypoxia ischemia, and microbial dysbiosis, all culminating in inflammation and gut injury [1–3]. Human milk, standardized feeding protocols, placental transfusion, and probiotics can decreased NEC risk [1–3].

In the University of Utah Medical Center (UUMC) Neonatal Intensive Care Unit (NICU), rates of NEC ≥ Bell stage 2 in infants born < 30 weeks gestational age (GA) increased from 5 to 11% from 2014 to 2015. This occurred after a decline from 21% to 5% from 2010 to 2014, associated with an umbilical cord milking (UCM) protocol introduced in 2011 and availability of pasteurized donor human milk (PDHM) in 2013 [4]. From June 2013 to September 2016, UCM protocol compliance was 93% and PDHM and mother's own milk use was 90–94%.

Intestinal microbial dysbiosis is associated with NEC [5–7] and can be modified by probiotics, defined as live microorganisms that confer a health benefit to the host [8]. As current evidence supports probiotic use in preventing NEC [7, 9, 10], our group determined there was no equipoise in studying probiotics in a randomized controlled fashion. Thus, in October 2016, we implemented a quality improvement (QI) protocol for probiotic use in infants born <$33^{0/7}$ weeks GA or <1500 g to decrease NEC rates. Our aim was to achieve a 50% reduction in NEC ≥ Bell Stage 2 in this population by October 2018. This equates to a change in rate from 0.14 to 0.07 per 100 patient days, or an annual rate of 7 to 3.5%.

Methods

Context

The UUMC NICU is a 52 bed, primarily inborn, academic, level III unit, admitting approximately 185 neonates <$33^{0/7}$ weeks GA per year. It is staffed by 21 attending

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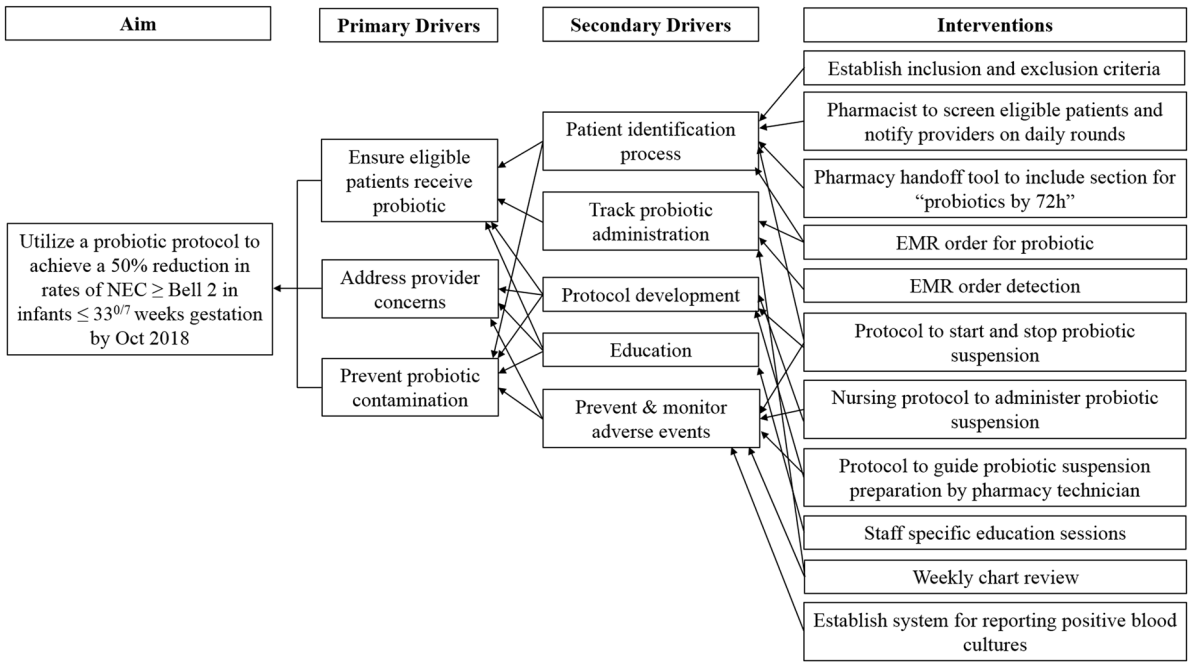

Fig. 1 Driver diagram. NEC necrotizing enterocolitis, EMR electronic medical record

neonatologists, 9 neonatal–perinatal medicine fellows, pediatric interns, 15 neonatal nurse practitioners, 140 nurses, 1 dietician, and 2 neonatal pharmacists. Bedside rounds are conducted daily and a NICU specialized pharmacist is present on rounds for most patients. Patients with surgical or multiple subspecialty needs are transferred to a level IV unit, which is staffed by the same neonatologists and fellows.

NEC risk is influenced by unit practices related to feeding, intestinal perfusion, and antimicrobial use [2]. In our unit, infants born <34 weeks GA or <1800 g qualify for PDHM, if their mother's milk is unavailable. Lactation services are available to all mothers of neonates in the NICU. There is a protocol for mothers separated from their infant to hand express breast milk within two hours of delivery, unless the mother cannot for medical reasons. A lactation consultant routinely audits this protocol. There was no standardized feeding protocol when this project began in October 2016, but one was introduced in July 2017. Enteral feeds are fortified with a bovine human milk fortifier.

Abnormalities in intestinal perfusion are also involved in NEC pathogenesis [1, 2]. An UCM protocol for infants born < 30 weeks GA was introduced in September 2011, the results of which are published [4, 11]. Ibuprofen and/or acetaminophen are typically used for medical closure of a symptomatic patent ductus arteriosus (PDA). However, there is no standardized protocol for diagnosing and treating a symptomatic PDA. Regarding antibiotic use, infants under an infectious "rule out" receive antibiotics for 48 h. Those undergoing treatment for bacteremia or pneumonia typically receive antibiotics for 7–10 and 5–7 days, respectively, though there is no standardized protocol for antibiotic use.

Intervention

Literature review guided protocol development and probiotic product selection. The Institute for Healthcare Improvement model for improvement guided project design. A driver diagram (Fig. 1) identified drivers and interventions necessary to achieve our aim.

Ultimate Flora Baby Probiotic (Renew Life, Palm Harbor, FL, USA) was selected due to the presence of multiple strains and quality assurance. This product contains four bifidobacteria (*Bifidobacterium breve HA-129, bifidum HA-132, longum subspecies infantis HA-117*, and *longum subspecies longum HA-135*) and *Lactobacillus rhamnosus HA-111*, for a total of 4 billion colony forming units (cfu) per gram. Ultimate Flora, branded as Flora Baby in Canada, was associated with a reduction in NEC in two Canadian cohort studies [12, 13]. Regarding quality, Ultimate Flora is manufactured in Canada, where it is subject to Natural Health Products Regulations under Health Canada's Natural and Non-prescription Health Products Directorate [14]. This includes licensing and labeling requirements, Good Manufacturing Practice standards, which are measures that ensure product quality and safety, and a natural product number (80020959 for Ultimate Flora) [14].

Table 1 shows our probiotic protocol. The inclusion and exclusion criteria are based on literature review showing no

Table 1 Probiotic protocol

Eligibility criteria	Discontinuation criteria	Re-start criteria
(a) Born <$33^{0/7}$ weeks GA or birth weight <1500 g	(a) Corrected GA $36^{0/7}$ weeks	(a) NPO for NEC: re-start once the patient is receiving 100 ml/kg/day enteral feeds
(b) Post-menstrual age ≥$24^{0/7}$ weeks	(b) NPO	(b) NPO for non-NEC issue: re-start once enteral feedings are resumed
(c) At least 72 h of age	(c) Ordered by a medical provider	
(d) Tolerating ≥6 ml/day enteral feeds for 24 h		
(e) No lethal anomalies/conditions or significant gastrointestinal anomalies		

GA gestational age, *NPO* null per os, *NEC* necrotizing enterocolitis

significant NEC reduction with probiotic use at older GA's, a time lag between birth and the start of probiotics, and probiotics being given with minimal enteral feeds. In our unit, the probiotic is treated like a medication. It is ordered in our electronic medical record (EMR), appears under the medication section in the patient's orders, doses are scanned prior to administration and can be tracked, and it is prepared and dispensed through the pharmacy. Our pharmacist is responsible for screening infants for probiotic eligibility or discontinuation (Table 1). A pharmacy technician prepares the probiotic suspension by mixing 500 mg of probiotic powder with 2 ml of D5W, for a total of 2 billion cfu per dose given once daily. The bedside nurse administers the probiotic suspension.

We implemented various measures to decrease the risk of probiotic sepsis. These included suspension preparation outside of patient care areas, use of gloves when handling the suspension, and requiring nursing staff to perform hand hygiene after probiotic administration. We also ensured our microbiology lab could culture *Lactobacillus* and *Bifidobacterium* species from standard pediatric blood culture specimens, if probiotic sepsis occurred.

We had four plan-do-study-act (PDSA) cycles. The first involved infrastructure design, which centered on protocol development, integration of the probiotic into our EMR, minimizing the risk of probiotic sepsis, and data tracking (Fig. 1). The second PDSA cycle was education and consensus building amongst all providers and ran concurrently with the first PDSA cycle. Provider targeted sessions were held to achieve buy in and educate providers on the protocol. We implemented the probiotic protocol for our third PDSA cycle, which began on October 3, 2016. The fourth cycle was intervention sustainment. During this cycle, we shared our data with providers and decided to continue our probiotic protocol.

Institutional review board approval was obtained from the University of Utah. The committee approved a waiver of informed consent, as probiotics were adopted as standard of care in our unit and this was a QI project.

Measures

Admission rates and rates of NEC ≥ Bell Stage 2 in infants born <$33^{0/7}$ weeks GA or <1500 g were followed. NEC staging was defined per the modified Bell's staging criteria [15]. Clinical presentation and operative findings differentiated surgical NEC from spontaneous intestinal perforation (SIP) [16]. We also reviewed the diagnosis assigned by the neonatologist and for surgical cases, neonatologist, surgeon, and pathologist, if a surgical specimen was available. We tracked cases of NEC through routine chart review and by establishing a system for our team lead to be notified of NEC cases in our unit.

Our primary aim was the monthly rate of NEC ≥ Bell Stage 2 per admissions of infants <$33^{0/7}$ weeks GA or <1500 g. However, this measure does not account for a patient's daily risk for NEC. Thus, retrospectively, we also measured monthly rates of NEC ≥ Bell Stage 2 per 100 patient days of infants <$33^{0/7}$ weeks GA or <1500 g. This measure is similar to that reported by Nathan et al. [17]. Infants transferred to the level IV unit for suspected intra-abdominal pathology were followed to identify cases of NEC diagnosed shortly after transfer, as excluding these cases would falsely lower our NEC rate. Finally, as a secondary aim, we measured monthly rates of surgical NEC and rates of NEC ≥ Bell Stage 2 in infants <$30^{0/7}$ weeks GA or <1500 g, to target a higher risk population.

Our process measure was protocol compliance, measured as the number of infants who received the probiotic per those eligible for the probiotic. Chart review and an EMR report function was used to monitor compliance. Our balancing measure was rates of probiotic sepsis, defined as a blood culture growing one or more of the organisms in our probiotic product. This was measured as cases of probiotic sepsis per infants receiving the probiotic. An automatic microbiology reporting system to the NICU medical director for positive cultures from neonates in our unit was already in place when this project began. Thus, we established a system for our team lead to be notified of blood cultures growing *Bifidobacterium* or *Lactobacillus* species.

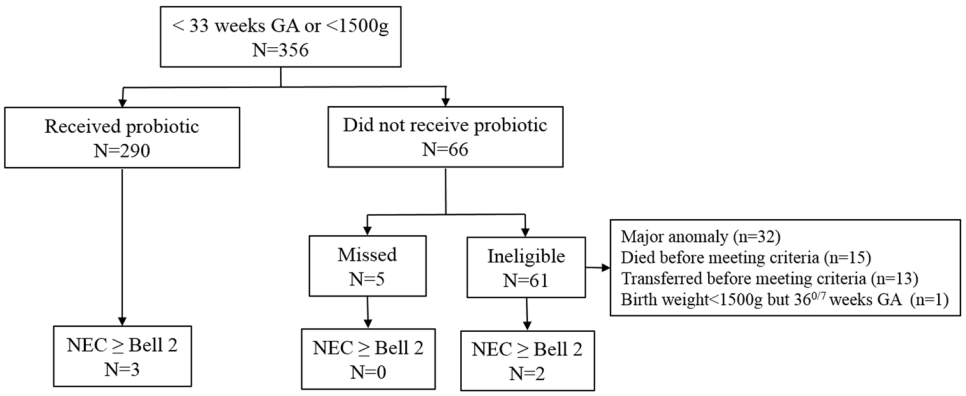

Fig. 2 Patient flowchart. GA gestational age, NEC necrotizing enterocolitis

Analysis

Outcome measures were analyzed with statistical process control (SPC) charts (QI Macros for Excel, version 2018, Denver, CO, USA). Montgomery SPC rules identified special cause change and set control limits as ±3 sigma lines [18, 19]. Monthly rates of NEC ≥ Bell stage 2 per admissions of infants born < $33^{0/7}$ weeks GA or <1500 g were plotted on a u chart. Monthly NEC ≥ Bell stage 2 per 100 patient days was plotted on a u' chart using Laney corrected control limits [20], to address potential over dispersion with large denominators. Categorical variables were analyzed by Chi-square or Fisher's Exact test. Student's *t*-test was used for analysis of normally distributed continuous data and Mann–Whitney *U*-test was applied for ordinal data or continuous data that was not normally distributed. Two-sided *p*-values < 0.05 were considered statistically significant. Logistic regression analysis was applied to determine the independent significance of risk factors on NEC. Statistical analysis was performed using SPSS (version 24, IBM, Armonk, NY, USA).

Results

Figure 2 shows the patient flowchart. Of 356 infants meeting GA and birth weight criteria, 295 (83%) met eligibility criteria for the probiotic. This represents 16,341 of 16,634 eligible patient days. Our protocol compliance rate was 98%, with 290 patients, representing 16,233 patient days, receiving the probiotic. Five (2%) eligible patients did not receive the probiotic. None of these infants developed NEC. These infants were missed, because they did not meet the GA criteria, but had a birth weight <1500 g. Once this was discovered, the NICU pharmacists were reminded of the birth weight criteria in the probiotic protocol. One ineligible infant received the probiotic. This infant was diagnosed with a cardiac anomaly on day of life 28. The probiotic was discontinued after diagnosis and this infant had no probiotic-related complications.

There were no significant differences in GA, birth weight, SIP, culture positive sepsis, and death in the pre-probiotic (October 2014–September 2016) and probiotic (October 2016–October 2018) periods (Supplementary Table 1). Medical treatment of PDA's was significantly different (10% pre-probiotic versus 5% post-probiotic, $p = 0.005$). Logistic regression analysis including GA, birth weight, probiotic exposure, and PDA treatment showed a significant effect of probiotics (odds ratio (OR) 0.21, 95% confidence interval (CI) 0.07–0.61) and PDA treatment (OR 0.38, 95% CI 0.15–0.96) on NEC. The rate of NEC ≥ Bell Stage 2 in infants born <$33^{0/7}$ weeks GA or <1500 g decreased from 0.14 to 0.04 per 100 patient days (Fig. 3a). This is equivalent to a decrease in annual rate from 7% to 2% (Fig. 3b). For infants born at <$30^{0/7}$ weeks GA or <1500 g, the rate of NEC ≥ Bell Stage 2 decreased from 0.15 to 0.03 per 100 patient days, or from 10% to 2% per year. The rate of surgical NEC in infants <$33^{0/7}$ weeks GA or <1500 g was unchanged. June 2017 was the point of special cause change (Fig. 3a, b).

There was a special cause increase in NEC in November 2017 (Table 2, cases 1 and 2). These cases were investigated and there were no changes in practice. There were five cases of NEC ≥ Bell Stage 2 in the probiotic period. As shown in Table 2, 3 of 5 cases were receiving probiotics at the time of NEC diagnosis. 1 infant transferred to the referral center for evaluation of pneumoperitoneum was diagnosed with NEC upon exploratory laparotomy and surgical pathology. No other patient was diagnosed with NEC after transfer. The median age of NEC diagnosis in the pre-probiotic period was 20 days (interquartile range (IQR) 14–28 days) versus 11 days (IQR 5–15 days) in the probiotic period, though this was not statistically significant ($p = 0.062$).

There were no cases of probiotic sepsis. However, there was one case of *Lactobacillus paracasei* and *Candida lusitaniae* sepsis occurring in a patient born at $26^{1/7}$ weeks GA who received three doses of the probiotic. This patient had a polymicrobial liver abscess, presumably due to a

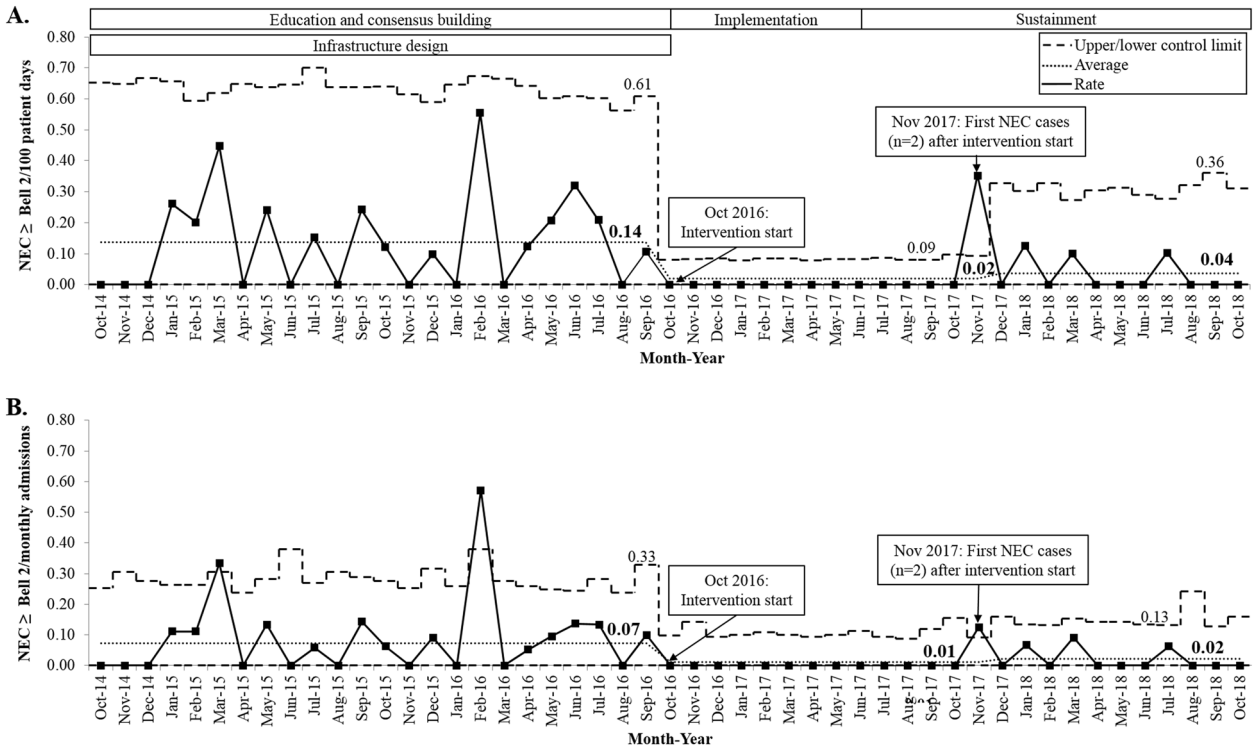

Fig. 3 Rates of NEC ≥ Bell Stage 2 in infants born <33$^{0/7}$ weeks GA or <1500 g shown as (**a**) U′ chart per 100 patient days with Laney-corrected control limits and (**b**) U chart per monthly admissions with upper and lower control limits at ±3 sigma lines. NEC necrotizing enterocolitis

Table 2 Characteristics of NEC Bell Stage ≥ 2 cases in the probiotic period

GA (weeks)	Birth weight (g)	Age at diagnosis (days)	Stage	Death due to NEC	On probiotic at diagnosis
25$^{5/7}$	965	15	Surgical	Yes[a]	Yes
28$^{2/7}$	520	11	Surgical	No	Yes
28$^{5/7}$	1030	3	Surgical	No	No[b]
26$^{2/7}$	705	8	Bell 2	No[c]	No[b]
32$^{0/7}$	2010	16	Bell 2	No	Yes

GA gestational age, *NEC* necrotizing enterocolitis

[a]Withdrawal of life sustaining support after open laparotomy revealed NEC total is associated with high volume packed red blood cell transfusion over a shortened period of time

[b]Infants did not meet the minimum feeding criteria prior to NEC diagnosis

[c]Withdrawal of life sustaining support occurred at 61 days of life after infant developed a second case of NEC, which was NEC totalis

bowel perforation, though he did not undergo an exploratory laparotomy to confirm this. This infant survived to discharge.

Discussion

The implementation of a probiotic protocol in this QI project was associated with a 75% reduction in rates of NEC ≥ Bell Stage 2 in infants born <33$^{0/7}$ weeks GA or <1500 g. Our rate of NEC ≥ Bell Stage 2 in this population decreased from 0.14 to 0.04 cases per 100 patient days. A similar decrease was seen in infants <30$^{0/7}$ weeks GA or <1500 g (0.15–0.03 cases per 100 patient days). There were no cases of probiotic sepsis.

Factors contributing to the success of this project included provider buy in, informatics support to build probiotic orders and monitoring reports, and treating the probiotic like a medication. Most importantly, our NICU pharmacist's role in identifying eligible patients was key to our high compliance rate, as they are present on bedside rounds daily and are responsible for reviewing patient medications. Furthermore, assigning the pharmacist oversight responsibility allowed for sustainability of our intervention without

multiple periods of re-education or re-training, as can be seen with providers on short-term rotations. It also alleviated the burden of remembering the probiotic protocol from providers.

Careful consideration was given to safety when designing our protocol and selecting the probiotic. Review of case reports of probiotic sepsis [21–26] allowed us to identify factors at the level of the patient, product, and unit that could contribute to probiotic sepsis. Patient level factors included a compromised intestinal epithelial barrier due to malformations, hemodynamic instability, or NEC. Probiotic product considerations included product contamination and less than the advertised number of cfu per gram of product. Finally, unit level concerns involved probiotic contamination of patient equipment, such as central lines and isolettes. To mitigate these risks, only infants without anomalies may receive the probiotic. Additionally, administering the probiotic to infants who are at least 72 h and tolerating trophic enteral feedings ensured that hemodynamically unstable infants or those with significant feeding intolerance did not receive the probiotic. Product quality was assured through regulations stipulated by Health Canada's Natural and Non-prescription Health Products Directorate; though neither Health Canada nor the United States Federal Drug Administration have approved any probiotic for the prevention of NEC. Finally, preparing the probiotic outside patient areas prevented potential probiotic aerosolization and nurses were educated on probiotic administration to prevent bed space contamination.

Our results are in line with multiple studies showing that probiotics prevent NEC. Randomized controlled trials of Infloran (*Bifidobacterium bifidum* and *Lactobacillus acidophilus*) [27] and ABC Dophilus (*Bifidobacterium infantis*, *Streptococcus thermophilus*, *B. bifidum*) [28, 29] in very low birth weight (VLBW) infants have shown a significant reduction in NEC ≥ Bell Stage 2 in the probiotic group. Moreover, a meta-analysis of 23 randomized controlled trials of probiotic supplementation to prevent NEC in VLBW infants showed an almost 50% reduction in NEC ≥ Bell Stage 2 with the use of probiotics [9]. Subgroup analysis of the probiotic type and NEC ≥ Bell Stage 2 showed a significant reduction in NEC when *Bifidobacterium* and *Lactobacillus* species were used in combination [9]. Finally, Janvier and colleagues' cohort study showed a significant reduction in NEC incidence from 9.8% to 5.4% with the use of the Flora Baby probiotic, branded as Ultimate Flora in the US [12]. This reduction in NEC occurred despite not having PDHM [12]. Similarly, Singh et al. showed a significant reduction in the probiotic group for the outcomes of NEC, mortality, and NEC and mortality [13]. Most infants in their probiotic group received Flora Baby.

We chose a multi-organism probiotic, because studies using multi-organism products show a significant reduction in NEC [12, 27, 28] versus those using a single strain [30, 31], with strains of *Bifidobacterium* and *Lactobacillus* used most often [7]. However, there are no randomized controlled trials comparing single versus multi-species probiotic products for NEC prevention. Moreover, meta-analyses have shown conflicting results when analyzing the effect of single versus multi-species products for NEC prevention. Thomas et al. showed a significant reduction in NEC only when using combinations of *Bifidobacterium* and *Lactobacillus* species [9]. In contrast, Sawh et al. showed a reduction of NEC with *Bifidobacterium* and *Lactobacillus* alone and in combination [32]. Similarly, a 2014 Cochrane review showed significant reductions in NEC with combination probiotic and those with only *Lactobacillus* species [10].

Limitations of our project include generalizability, as it was conducted in a single center, which may limit applicability to units with different microsystems. However, elements of our protocol can be adopted by other units, such as patient selection criteria, strategies to minimize the risk for probiotic sepsis, and having personnel in the NICU on a daily basis in charge of administering the protocol (e.g. pharmacist in our unit). 87% of infants admitted to our NICU between October 2014 and October 2018 received their mother's own milk. In contrast, the median rate of mother's own milk use is 75% (IQR 60–86%) across the United States [33]. Thus, the effect of probiotics on NEC risk could differ in units with lower rates of mother's milk usage. However, Bin-Nun and Lin et al.'s randomized controlled trials showed significant reductions in NEC, despite having exclusive mother's milk use rates of 58% and 69%, respectively [27, 28]. Another limitation to our results is the introduction of a feeding protocol in July 2017, 9 months after the probiotic protocol. Feeding protocols can decrease NEC rates by limiting variability in feeding practices amongst providers [3, 17]. However, special cause change was demonstrated prior to the introduction of the feeding protocol. Therefore, it is unlikely that the feeding protocol accounted for the reduction in NEC rates seen in our unit. In addition, PDA treatment was a significant risk factor for NEC. Decreased PDA treatment unlikely contributed to decreased NEC rates in our population, as recent evidence suggests that neither conservative nor medical treatment of a PDA is associated with NEC [34–36]. Finally, limitations to the Bell's staging criteria for NEC [37] are highlighted by NEC diagnosis in two patients receiving minimal enteral feedings (cases 2 and 3, Table 2) and a trend toward an earlier median age of NEC in the probiotic period.

In regards to probiotic sepsis, it is possible that cases of probiotic sepsis were missed, as our protocol did not require providers to obtain anaerobic cultures in infants undergoing a sepsis evaluation. In the one case of *L. paracasei* and *C.*

lusitaniae sepsis in an infant with a polymicrobial liver abscess, there is a chance the *Lactobacillus* species was misidentified. *L. rhamnosus*, which is in our probiotic product, and *L. paracasei* are subspecies of *Lactobacillus casei*, and could therefore be difficult to distinguish from each other by culture alone [38]. However, the bowel was presumably compromised, given the presence of a polymicrobial liver abscess with organisms colonizing the gastrointestinal tract. This highlights the importance of probiotic use in infants with an intact intestinal epithelial barrier, as we attempted to do in our protocol's inclusion and exclusion criteria.

In conclusion, this QI project involving implementation of a probiotic protocol was associated with a reduction in rates of NEC ≥ Bell Stage 2. Key drivers of success were widespread buy in, assigning a point person with the task of screening eligible patients, informatics support to build a probiotic monitoring report, and monitoring for adverse outcomes. Additional single and multicenter QI projects are necessary to evaluate optimal methods of probiotic protocol implementation in preterm infants.

Author contributions MKS was involved in probiotic protocol implementation, compliance tracking, data collection and analysis, and manuscript preparation. PHG assisted in data analysis and manuscript revision. MN was involved in data extraction from the EMR. BAY developed the probiotic protocol, monitored compliance, and reviewed the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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