

Cohort Study of Probiotics in a North American Neonatal Intensive Care Unit

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Objective To determine whether routine probiotic administration to very preterm infants would reduce the incidence of necrotizing enterocolitis (NEC) without adverse consequences.

Study design Since the end of July 2011, we have administered a probiotic mixture to all admitted infants of <32 weeks' gestation. We give 0.5 g of a mixture of 4 bifidobacteria (*Bifidobacterium breve*, *bifidum*, *infantis*, and *longum*) and *Lactobacillus rhamnosus* GG (2×10^9 colony-forming units) per day, starting with the first feed, until the infant reaches 34 weeks. We compared complications among infants admitted during the first 17 months of routine use with those admitted during the previous 17 months.

Results Two hundred ninety-four infants received probiotics, and 317 infants formed the comparison group. Introduction of probiotics was associated with a reduction in NEC (from 9.8% to 5.4%, $P < .02$), a nonsignificant decrease in death (9.8% to 6.8%), and a significant reduction in the combined outcome of death or NEC (from 17% to 10.5%, $P < .05$). After adjustment for gestational age, intrauterine growth restriction, and sex, the improvements remained significant (OR for NEC, 0.51; 95% CI, 0.26-0.98; OR for death or NEC, 0.56; 95% CI, 0.33-0.93). There was no effect of probiotics on health care-associated infection.

Discussion A product that is readily available in North America, that has excellent quality control, and that contains strains similar to those that have been shown effective in randomized controlled trials substantially reduced the frequency of NEC in our neonatal intensive care unit. (*J Pediatr* 2014; ■: ■-■).

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There are now >22 randomized controlled trials (RCTs) of probiotic preparations in preterm infants, enrolling >5000 infants.¹⁻¹⁵ Systematic reviews¹⁶⁻¹⁸ of these trials have documented a major reduction in necrotizing enterocolitis (NEC) and a reduction in death with the routine use of probiotics in high-risk preterm infants. No significant adverse consequences of probiotic administration have been documented in the RCTs. Subgroup analyses demonstrate little difference in the effects of probiotics between those containing lactobacilli, those containing just *Bifidobacteria*, and those containing a mixture, although there are trends suggesting that a mixture of different organisms may be more effective than a single species.¹⁹

In Canada, probiotics sold as health-promoting agents are under the jurisdiction of the Natural Health Products Directorate, which has rigorous requirements for quality control and detailed labeling. To have a natural product number, the manufacturer must abide by Good Manufacturing Practice, in registered and inspected production facilities, have stringent quality control, and register the DNA of the organisms in a Health Canada database. No preparation of probiotic is licensed by Health Canada for the prevention of NEC. In 2011, faced with overwhelming evidence that probiotics could decrease NEC in preterm infants, and because there were no significant risks described in the extensive literature, we decided to introduce probiotics as routine care for the prevention of NEC. Before administering the probiotics, our laboratory personnel confirmed that they were able to grow the organisms in routine culture and perform generic identification confirming the presence of *Lactobacillus* and *Bifidobacteria*, but identification of the precise strain is not possible. In addition, a provincial service is available for further DNA analysis if necessary. We examined the clinical consequences of the introduction of probiotics in our neonatal intensive care unit (NICU) and hypothesized that serious cases of NEC would decrease following their introduction.

GI	Gastrointestinal
HCAI	Health care-associated infection
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
RCT	Randomized controlled trial
SGA	Small for gestational age

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Methods

We performed a prospective cohort study of infants receiving probiotics in our NICU. After the first 17 months of use, we compared the incidence of NEC and death with that during the final 17 months before the introduction of probiotics. We decided a priori that a period of 17 months before and after the introduction of probiotics should give a sample of about 300 eligible infants per group, given our usual frequency of admission of very preterm infants, and therefore 80% power to determine if there was a 60% reduction in NEC (from 10% to 4%, similar to the relative risk described in the systematic reviews) with a *P* value of .05.²⁰⁻²²

All infants admitted to the NICU at Sainte Justine University Health Center have their medical information recorded in the Canadian Neonatal Network Database. This information was used to identify infants for chart review. The Institutional Review Board of Sainte Justine approved both the anonymized prospective data collection and retrospective chart review. The study is reported following the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.²³

Since July 2011, all infants <32 weeks' gestation at birth were eligible to receive probiotics unless they had a gastrointestinal (GI) malformation. They received FloraBABY (Renew Life Canada, Oakville, Ontario, Canada) probiotic mixture; this was chosen because it has a Health Canada Natural Product Number and contains 4 *Bifidobacteria* (*Bifidobacterium breve*, *bifidum*, *infantis*, and *longum*) and *Lactobacillus rhamnosus* GG (2×10^9 colony-forming units per 0.5 g) mixed with 1 mL of water just before a milk feed once a day. Probiotics were started at the time of the first feed and continued until the infant reached 34 weeks' postmenstrual age. Infants who were receiving probiotics and developed NEC had probiotics discontinued for the period of being nil by mouth, and restarted afterward, in the hope of preventing recurrence of NEC. Probiotics were continued during sepsis or other acute illnesses unless the infant was placed nil by mouth.

Infants admitted to our NICU during the last 17 months before commencing probiotics, January 2010 to May 31, 2011, were included in the comparison group. We included all infants <32 weeks' gestation who were born at Sainte Justine or were transferred from another center before 3 days of age. Among data abstracted, we included gestation, sex, intrauterine growth restriction (ie, <10th percentile), having an umbilical catheter, being ventilated for at least the first 3 days of life, and completion of antenatal steroids.

In June and July 2011, administration of probiotics was not routine; 6 infants received probiotics, which were started after an episode of feeding intolerance or stage 1 or stage 2 NEC. Therefore, we did not include any data from infants born during June or July 2011. The protocol for routine administration was introduced in the last week of July 2011, and all infants <32 weeks' gestation admitted during the 17 months between August 1, 2011, and December 31, 2012, were included.

Outcome Variables

NEC was diagnosed on the basis of a combination of clinical signs and abdominal radiography and classified according to the modified Bell classification—stage 2 or 3 was considered definite NEC for the purposes of this study. The radiologists' reports were reviewed, and only those infants with a radiographic diagnosis of pneumatosis, portal venous air, or perforation were included as cases of NEC. The radiologists were unaware of the start of probiotic use in our NICU. Two infants had surgery despite not having other radiologic criteria for definite NEC; both had confirmatory pathology and are defined as being NEC. Mortality was defined as death before final discharge home. Infants presenting with typical clinical features of spontaneous intestinal perforation were not included as cases of NEC, unless the diagnosis was changed at the time of surgery.

Health care–associated infection (HCAI) was defined by a positive culture of a normally sterile body fluid (blood, cerebrospinal fluid, peritoneal fluid), occurring after 3 days of age. Length of stay was defined from date of birth until day of final discharge home. The day when probiotics were first actually received was recorded, as was the day they were stopped.

As a proxy for feeding tolerance, we recorded the day that intravenous nutrition was first stopped and the final day of receiving intravenous nutrition, including periods if it was restarted.

All infants born during the 2 study periods were included, including a few in the probiotic group who never received probiotics, either because of death or NEC occurring before the commencement of probiotics, or a few more mature infants (mostly of 31 weeks' gestation) who were not treated according to protocol, due to oversight.

Other Characteristics of Practice

About 1 year before the start of the first cohort, a new feeding protocol, designed to encourage the use of maternal breast milk and to rapidly advance feeds in preterm infants, was introduced in our NICU. There was no change in the protocol during the subsequent 4 years. Donor breast milk was not available during the entire study period; when breast milk was not available, infants received formula. The feeding protocol has guidelines for feeding in the presence of umbilical catheters and for feeding during treatment of a patent ductus arteriosus, which did not change during the study period.

Use of blood transfusions is not protocolized in our NICU, being prescribed according to perceived individual need by the attending staff, who did not change their approaches during this period.

Statistical Analyses

Data were analyzed initially by descriptive statistics. Initial comparison of the frequency of NEC and death and other categorical and continuous variables in the 2 groups used the Fisher exact test for proportional data and unpaired *t* tests

for continuous variables. Further evaluation of the data involved multiple logistic regression to correct for risk factors between the groups.

Results

All infants admitted to our NICU before 48 hours of age and with a gestational age of <32 weeks were included in our analyses, except for a single infant in the postprobiotic cohort, who had gastroschisis. All infants who were transferred to other level II hospitals before discharge home ($n = 105$) survived without being readmitted to our hospital with NEC. There were 13 infants in the probiotic cohort period who did not receive probiotics; their data were included in the analysis of the probiotic cohort.

Historical Comparison Cohort

During the 17 months before the introduction of probiotics, we admitted 317 infants of <32 weeks' gestation to our NICU. Their mean birth weight, gestational age, and other characteristics are shown in [Table I](#). The incidences of NEC, HCAI, and death are shown in [Table II](#).

Probiotic Cohort

Two hundred ninety-four infants of <32 weeks' gestational age were admitted in the probiotic cohort ([Table I](#)). There was a significant reduction in the incidence of NEC, a nonsignificant reduction in death, and a significant reduction in the proportion of infants who either died or developed NEC in comparing periods 1 and 2 ([Table II](#)). There was no significant effect on HCAI. The average duration of therapy was 24 (SD 16) days, and the average day of starting probiotics was day 4.3 (SD 5.1).

Restricting the analysis to only the infants with a birth weight of <1001 g showed very similar percentage reductions in NEC and the combined outcome of death and NEC. The study did not have enough power for these subgroup effects to be significant ([Table III](#)).

There was no significant difference in average gestational age between the control and the probiotic cohorts, or in the proportion who were small for gestational age (SGA), or the proportion who were female or <26 weeks' gestation ([Table I](#)). Nevertheless, we performed a logistic regression analysis, correcting for gestational age, being SGA, sex,

Table I. Characteristics of infants included in the 2 cohorts

Characteristic	Preprobiotic cohort (n = 317)	Probiotic cohort (n = 294)
Gestational age, mean wk (SD)	28.9 (2.2)	29.0 (2.1)
Infants <26 wk' gestational age	13.2%	11.1%
Birth weight, mean g (SD)	1207 (376)	1220 (334)
SGA (<10th percentile)	11.4%	16%
Partial or complete antenatal steroid use	84%	82%
Female	44%	51%

Table II. Clinical outcomes of infants in the 2 cohorts

Outcome	Preprobiotic cohort (n = 317)	Probiotic cohort (n = 294)	Significance (P)
NEC	31 (10%)	16 (5%)	<.05
Death	31 (10%)	20 (7%)	NS
NEC or death	54 (17%)	31 (11%)	<.05
HCAI	57 (18%)	54 (17%)	NS
Diagnosis of NEC, mean d of life (SD)	22 (20)	18 (11)	NS
NEC before 15 d of life, No. (percentage of NEC cases)	11 (35%)	8 (50%)	NS
Infants receiving at least 1 day of TPN, No.	295 (94%)	282 (96%)	NS
Mean age at first stopping TPN, d (SD)	16 (20)	11 (10)	.004
Mean age at finally stopping TPN, (SD)	22 (28)	16 (18)	.02

NS, nonsignificant; TPN, intravenous nutrition.

having an umbilical arterial catheter, and being ventilated >3 days of life. Being ventilated for >3 days and having an umbilical catheter were not significant for any of the outcomes and therefore were deleted from the model. [Table IV](#) shows the ORs and 95% confidence intervals for probiotic therapy, and the other important risk factors on the outcomes of NEC, death, NEC or death, and HCAI.

The proportion of mothers in both periods who provided breast milk during the first month of life of the infants was unchanged (91% of historical comparison group and 93% of probiotic group). The age at diagnosis of NEC was not significantly lower after introducing probiotics, and the proportion of early cases, in the first 2 weeks of life, was increased but not significantly ([Table II](#)).

The reduction in NEC from approximately 10% to 5% meant that the number needed to treat to prevent 1 adverse outcome (death or NEC) in our NICU was 20. There was no effect of cohort on the incidence of HCAI, either before or after adjustment.

The duration of intravenous nutrition among the infants who received it for at least 1 day was significantly shorter in the probiotic period ([Table II](#)); the mean difference in the time to first stopping intravenous nutrition was 4.6 days (95% CI 1.8-7.3 days), and the mean difference in time to finally stopping intravenous nutrition (which included any episodes of restarting intravenous nutrition) was 5.3 days (95% CI 0.8-9.9 days).

Discussion

Probiotics are "live micro-organisms which when administered in adequate amounts confer a health benefit on the host." The intestine of the normally delivered term, breast-fed infant is rapidly colonized with a number of probiotic organisms, in particular *Bifidobacteria* and lactobacilli, but the precise pattern and species involved differ around the world. In contrast, the preterm newborn intestine tends to be colonized by different microorganisms, predominantly

Table III. Results among infants <1001 g at birth

Outcome, No.	Preprobiotics (n = 109)	Probiotics (n = 98)	Significance (P)
NEC	18 (17%)	10 (10%)	NS
Death	27 (25%)	14 (14%)	NS
NEC or death	38 (35%)	22 (22%)	NS
HCAI	38 (35%)	30 (30%)	NS

coliforms, enterococci, and other bacteroides species, with much interindividual variation.²⁴ Changes in the intestinal microbiome and shift to a predominance of Enterobacteriaceae before the onset of NEC have also been shown.²⁵

The mortality and long-term GI, neurologic, and developmental morbidity rates of NEC are substantial. Preventing NEC is therefore a priority in the support of the very preterm infant. Administration of probiotic organisms as a means of preventing NEC is biologically feasible, is supported by extensive published clinical evidence, and has a clearly beneficial risk-benefit balance. As a result, in the summer of 2011, we introduced probiotics as routine prophylaxis in at-risk infants in our NICU. Although the use of probiotics has been routine for several years in many NICUs outside of North America, the administration of probiotics in North America has been limited.²⁶

The administration of live bacteria to immunoincompetent patients such as the very preterm infant cannot be taken lightly. Cases of probiotic-associated bacterial sepsis that have been reported have mostly been secondary to lactobacilli; 4 cases have been reported in young children, and all cases have been mild and easily treated.²⁷⁻²⁹ Several of these cases involved children with congenital intestinal anomalies. *Bifidobacteria* has been reported as causing sepsis in a newborn infant³⁰; a relatively mild illness occurred in an infant after surgery for omphalocele. An extensive review of the literature has concluded that "Current evidence suggests that the risk of infection with probiotic lactobacilli or bifidobacterium is similar to that of infection with commensal strains, and that consumption of such products presents a negligible risk to consumers, including immunocompromised hosts."³¹ The GI tracts of all preterm infants become colonized. Failure to give probiotics does not lead to a sterile GI tract; instead, in an NICU environment, the tract will otherwise become colonized with a variety of pathogenic organisms. The risk and morbidity of the very rare case of sepsis due to *Lactobacillus*,

which usually resolves with simple antibiotics, should, however, be weighed against the potential for colonization with much more pathological bacteria and the morbidity and substantial mortality rates following NEC. Furthermore, in the large number of published RCTs, now enrolling >5000 preterm infants, no cases of sepsis with probiotic organisms have been reported. Similarly, in our cohort, we had no cases of sepsis identified as being due to the probiotic organisms.

Our cohort showed a nonsignificant decrease in HCAI after the introduction of probiotics. This is consistent with the experience in the RCTs, which showed a reduction in sepsis of marginal statistical significance.¹⁶ Further attempts to reduce HCAI will require other approaches, such as perhaps the use of lactoferrin, which, from preliminary data, appears particularly effective in combination with probiotics.^{32,33}

As a prospective cohort study, with a historical comparison cohort, the study has several limitations. The incidence of NEC may vary from year to year, but in fact in our NICU the annual reports from the Canadian Neonatal Network have shown a relatively stable incidence from year to year, which has tended to be above the Canadian reference rate for each of the previous 6 years. Confounding of the results by other changes in practice is also a risk in this type of study. However, the only proven postnatal modifiable risk factors for NEC are the use of breast milk³⁴ and having a standardized feeding protocol.³⁵ The use of breast milk changed very little during this period, remaining at a high rate achieved through a previous very active quality improvement initiative, and an established feeding protocol was already in place before the start of the control period. We were unable to show an effect of the use of umbilical catheters or of early assisted ventilation (as indicators of severity of illness) on the incidence of NEC, but we did confirm that being SGA, male, and more immature increase the risk of NEC, and we adjusted our analysis for these factors. The radiologic diagnosis of NEC is also somewhat subjective,³⁶ especially for moderately severe cases, but the radiologists were not aware of the introduction of probiotics in our NICU, although not formally masked. Therefore, risks of bias from differential rates of diagnosis are low.

The reasons given for not using probiotics routinely for preterm infants have included the lack of mechanistic understanding, the lack of an available preparation, the preponderance of studies from units or networks with a high incidence, and the lack of studies from North America.^{26,37,38} However,

Table IV. Results of logistic regression analysis

Outcome	OR for risk factors (95% CI)			
	Receipt of probiotics	Gestational age (per additional week)	Being SGA	Being female
NEC	0.51 (0.26-0.98)*	0.72 (0.62-0.83)*	2.6 (1.1-5.8)*	0.44 (0.23-0.87)†
Death	0.71 (0.38-1.34)	0.56 (0.48-0.66)*	3.5 (1.54-7.84)*	0.80 (0.43-1.5)
NEC or death	0.56 (0.33-0.93)†	0.62 (0.54-0.69)*	3.8 (1.95-7.30)*	0.72 (0.43-1.19)
HCAI	0.98 (0.81-1.98)	0.56 (0.50-0.63)‡	1.73 (0.90-3.34)	0.83 (0.52-1.31)

*P < .05.

†P < .02.

‡P < .001.

probiotics are now known to be effective, despite a limited understanding of the exact mechanisms, and research in this area is advancing very rapidly. Although many studies have been from centers with a high incidence of NEC, there are also studies from lower-incidence populations,³⁹ and the recently completed and presented ProPrems trial⁴⁰ showed a significant, >50%, reduction in NEC despite an incidence in their control patients of only 4.4%. Finally, although not an RCT, the current study is from a large academic North American NICU, showing that the results in the RCTs are indeed translatable to our environment, using a preparation easily available in Canada and the US. Compared with other innovations in neonatology that have become standard of care, evidence demonstrating that probiotics are effective and safe is substantial.²⁶ Probiotics are different from these other innovations. It is inexpensive to manufacture the quantities required for a small preterm infant. Given the low cost of probiotics and the regulatory difficulties surrounding them,²⁶ the potential profits are quite small. Probiotic manufacturers do not have the financial incentive to conduct the rigorous trials that would be necessary to seek regulatory approval of probiotics as a drug.

We obtained FloraBABY from the suppliers at a cost of \$12.79 for a 60-g tub. Each infant is supplied with his or her own tub. As the number needed to treat is 20, this implies that the cost to prevent 1 death or case of NEC is about \$260. Because we do not wish to pass an opened tub of probiotics to another infant, the remainder is disposed of. However, as we give 0.5 g a day, the cost of the probiotic preparation actually administered to the infant (11 cents per day × 23.6 days) averages \$2.51, for a cost per NEC or death saved of \$50. A single-dose preparation would be an advantage, both for security of handling and for reducing waste and costs.

The improvement in feeding tolerance, as demonstrated by the reduction in the duration of intravenous nutrition, is another benefit that leads to substantial cost-savings; the reduced cost of intravenous nutrition could easily exceed the costs of the probiotics.

The overall numbers of cases of NEC in the US and Canada are not certain. The annual report of the Canadian Neonatal Network, which covers the large majority of very preterm births in Canada, reports around 250 total cases among 4000 very preterm infants added to the database each year. With almost 80 000 very preterm infants born in the US annually, one could estimate a total number of cases of around 4800 per year. This would imply that universal adoption of probiotic prophylaxis has the potential to prevent 2500 cases of NEC every year in North American neonatal units.

Just as in the 22 RCTs currently published, we showed substantial benefits of probiotics and no adverse events. A reliable preparation is easily available in Canada and the US. Of note, the recently completed ProPrems trial used a preparation called ABC Dophilus Probiotic Powder for Infants (Solgar, Leonia, New Jersey). Other reliable preparations are available around the world; good quality control and confirmation of the contents of the preparation are essential. Probiotics should be considered standard of care

and used routinely to all preterm infants of <32 weeks' gestation. Additional studies comparing strains and examining additional NICU populations and length of therapy are needed. There seems to be no further reason to delay the introduction of this evidence-based therapy in the NICU. ■

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