The introduction of multi-strain probiotics to preterm infants in a regional hospital: An observational study

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Background. Worldwide 1 in 10 infants is born preterm. Late-onset sepsis (LOS) and necrotising enterocolitis (NEC) are important causes of mortality and morbidity in this vulnerable group, especially in those with birthweights less than 1 500 g. Probiotics may help to decrease the incidence of these conditions, although controversies remain.
Objectives. To describe the introduction of multi-strain probiotics to infants with birthweights ranging from 800 to 1 200 g at a regional hospital, determine the incidence of NEC, LOS and mortality in this group, and to compare the findings with previous years when there were no probiotics or single-strain probiotics.
Methods. We conducted a retrospective observational study of infants who received probiotics at a regional hospital between February 2019 and July 2020. Outcome data, including mortality, NEC and LOS were collected. These data were compared with previous time periods.
Results. Seventy-seven infants were included. All infants who qualified for probiotics received them. They had a median (IQR) weight of 1 000 (900 - 1 200) g and a median (IQR) gestation of 30 (28 - 31) weeks. All infants received breastmilk. A total of 11 (14.3%) infants had positive blood cultures which were predominantly gram-negative organisms and there were no cultures of probiotic organisms. Infants with birthweights less than 1 kg contributed 75% of the sepsis episodes. There were seven deaths (9%) of which 3 occurred before 72 hours of life. Four of the deaths were attributed to LOS. There were no cases of NEC during this period. Compared with previous time periods, there were similar rates of LOS, but a reduction of NEC.
Conclusion. The introduction of probiotics to a regional hospital is possible. Less NEC was observed when multi-strain probiotics were used.

Keywords. probiotics; necrotising enterocolitis; preterm; VLBW; late-onset sepsis.

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Worldwide, ~1 in 10 infants is born preterm, i.e. <37 completed weeks of gestation, with the highest burden (of preterm births) in low- and middle-income countries (LMICs). Neonatal sepsis and necrotising enterocolitis (NEC) are two of the largest contributors to preterm neonatal mortality and morbidity, especially in those with very low birthweights (VLBW) (<1 500 g).[1]

Growing evidence supports the key role of a healthy gut microbiome in promoting and maintaining the gut barrier in the immediate postnatal life. In preterm infants (especially VLBW infants) development of the gut microbiome is disrupted by events related to prematurity, including mode of delivery, antenatal and postnatal use of antibiotics, minimal exposure to maternal flora as well as low breastmilk intake. Such disruption, called dysbiosis, results in an altered barrier and immune function and an imbalance between pro- and anti-inflammatory responses, which has been associated with NEC and late-onset sepsis (LOS).[2]

Neonatal sepsis is ranked as the third highest cause of neonatal deaths and has a high mortality in the newborn population, especially for preterm infants. Neonatal sepsis is a clinical syndrome consisting of nonspecific signs accompanied by bacteraemia.[3] LOS (after 72 hours of life) is more common than early-onset sepsis.[4] LOS are typically nosocomial infections with nonspecific features which include lethargy, feeding intolerance, glucose instability, irritability, temperature instability, bradycardia or tachycardia, poor perfusion and apnoea.[3] Antimicrobial resistance (AMR) has emerged as a global threat to healthcare, resulting in an increase in morbidity and mortality. An estimated 31% of deaths from neonatal sepsis are attributed to AMR. [5]

Measures focused on preventing sepsis of the newborn include the important pillars of hand hygiene, the reduction of invasive procedures and early enteral feeding. Breastmilk is the ideal natural way to help impart anti-infective, anti-inflammatory and immunomodulatory properties to the newborn.[6]

Necrotising enterocolitis

NEC is a disease seen primarily in preterm infants. It is a serious condition and results in significant morbidity and mortality. Approximately 5% (1.9 - 12.9%) of infants with a gestational age (GA) less than 33 weeks develop NEC and mortality rates of 30% occur even in the best-resourced units.[7] The exact cause is not understood. However, mucosal injury, destruction of bowel wall integrity, bacterial translocation and overgrowth trigger a severe and unregulated inflammatory immune response. These infants present with signs such as feeding intolerance, abdominal distention, and blood in the stools. They may have haemodynamic instability and may progress to bowel perforation and peritonitis. NEC can lead to fulminant sepsis and death.[8] It is staged by the modified Bell's staging system (Appendix A; http://coding.samedical.org/file/2349).[8] NEC rates vary between units and countries with higher rates reported in LMICs.[9]

Several risk factors for NEC have been identified including maternal, perinatal and infant factors. Maternal factors include

gestational diabetes, isoimmunisation, chorioamnionitis, gestational hypertension and fetal growth restriction. Perinatal factors include fetal distress and fetal hypoxia. Infant factors contribute the greatest risk for NEC. The incidence of NEC is inversely proportional to the GA and birthweight. Intra-uterine growth restriction, congenital heart disease, umbilical vascular access lines, polycythaemia, sepsis and exchange transfusion all pose risks.[10] Human breastmilk is well known to be protective against NEC as compared with formula.[6]

Most theories about the pathogenesis of NEC have focused on the most important risk factors, such as immaturity, formula feeding and the presence of bacteria. Most recently, gut dysbiosis has been proposed as the main risk factor for development of NEC. Based on this theory several clinical strategies are being recommended. These include breastmilk feeding, restrictive use of antibiotics, supplementation with probiotics and standardised feeding protocols.[11]

Probiotics

Probiotics are known to improve gut maturity and function in preterm infants.[12] Probiotics are defined as 'live microorganisms' which confer health benefits to the host. They are classified as a food supplement, rather than medication, which benefits the host by improving the intestinal microbial balance.

The administration of probiotics has been proposed as a potential tool to prevent NEC and LOS. Updated meta-analyses confirm the benefits of probiotics in reducing the risk of NEC, the time to achieve full enteral feeding, and the risk of LOS in preterm infants.[6] Most of the meta-analyses failed to explore the role of probiotics in detail and do not provide specific recommendations regarding which probiotic strain or mixture of strains should be used or which population(s) would benefit most from the use of probiotics.[6] However, there is consensus among them in that the use of multi-strain probiotics (especially *Lactobacillus* spp. and *Bifidobacterium* spp.) proved to be more effective than single strains.[13,14]

A 2020 Cochrane meta-analysis showed there was benefit in using probiotics to help reduce the risk of NEC, sepsis and mortality. However, when only studies with a low risk of bias were included, there was only a benefit in preventing NEC. The benefit for ELBW infants was not significant.[15]

High cost is a potential disadvantage of probiotic use, especially routine probiotic use. This is an extremely important factor and represents a large financial commitment in a resource-limited setting. Although multi-strain is considered better than single strain, there is no consensus regarding which strains represent the most beneficial combination. There is also limited research published on ELBW neonates.

George Hospital (GH) neonatal unit is the referral point for 9 level-one hospitals and in addition to secondary level care performs some tertiary level functions such as ventilation and therapeutic hypothermia. It has a milk bank and a policy of using only exclusive breastmilk (EBM) from the mother or donor EBM (*d*EBM) for all infants with a birthweight <1 500 g until their weight exceeds 1 500 g. All medical NEC cases are managed in GH with surgical referrals transferred to Red Cross War Memorial Children's Hospital (Cape Town, SA).

GH adopted a single-strain probiotic incorporating *Lactobacillus reuteri* (Reuterina Drops (Austell, South Africa)) in June 2017. In February 2019, the decision was made to change to multi-strain probiotics (Labinic Probiotic Drops (Biofloratech Ltd., UK)).

Labinic probiotic drops were introduced to South Africa in 2018. The 3 strains are present equal quantities in Labinic Drops,– *Lactobillus Acidophilus* (NCFM), *Bididobacterium bifidum* (Bb-01) and *Bifidobacterium infantis* (Bi-26). Each Labinic vial cost ZAR842 (USD52) in 2020.

All infants weighing 800 - 1 200 g and who were born during or after February 2019 were eligible for Labinic drops. The probiotics were administered (2 drops daily) from the first day of life, once a day, and continued until they reached a weight of 1 200 g.

Importance of study

The present study was important to determine if it was possible to properly implement the introduction of probiotics in a level-two hospital in an LMIC. It would also be important to measure if there was any associated decrease in NEC, LOS or mortality.

Objectives

The primary objectives of the study were to describe the administration of multi-strain probiotics and determine the incidence of NEC, LOS and mortality in this group.

Secondary objectives were to compare our findings with outcomes from GH from January 2016 to January 2020, where there were either no probiotics or single-strain probiotics.

Methods

This is a retrospective observational study. Inclusion criteria were all infants born from February 2019 till July 2020 with birthweights ranging from 800 g to 1 200 g. Infants with missing or incomplete records were excluded from the present study. Infants were identified from a probiotic register book where the demographics of all those who received probiotics were recorded. Folders of these infants were reviewed (all infant records in the neonatal unit are stored electronically). Case report forms were completed on Microsoft Forms (Microsoft Corp., USA). LOS sepsis was defined as infants (>72 hours of life) with a positive blood culture and NEC was defined as Bell's criteria of Stage 2 and above at any period during the admission in the unit. Mortality was documented as any deaths until discharge to home or transfer. LOS and NEC outcomes in this cohort were compared with the pre-probiotic and single-strain eras.

Results from this cohort of infants were then compared with two time periods when there were no probiotics (January 2016 - May 2017) and (only) single-strain probiotics (June 2017 - January 2020). This information was extracted from ward registers, the National Health Laboratory Service and the Perinatal Problem Identification Programme.

Statistics

Descriptive statistics were performed with numerical variables expressed as means and standard deviations (parametric) or median and IQR (non-parametric). Chi-squared and Fisher's exact tests were used to determine if there was a difference between categorical variables.

Ethics

Ethical clearance was obtained from Health Research and Ethics Committee of the University of Cape Town (ref. no. HREC 537/2022) and permission was obtained from the hospital management. Parental consent was waived owing to the retrospective nature of the study, as well as deidentification and pooling of data.

Results

Seventy-nine infants received probiotics. Two infants were excluded based on weight criteria (720 g and 780 g, respectively), therefore a total of 77 infants were included in the study. All folders were retrieved and all infants received probiotics.

Maternal characteristics

There were 75 mothers whose infants were included in the study; there were 3 sets of twins, with one twin weighing >1 200 g.

All the mothers received antenatal care prior to their deliveries. Fifty-nine (78.6%) mothers delivered via caesarean section. Sixty-five (86.6%) of the mothers received antenatal steroids. Two of the mothers tested positive for syphilis in pregnancy but were fully treated and their infants were asymptomatic. Thirteen mothers were RVD-reactive, but only one did not have antiretroviral drugs prior to delivery, as she was diagnosed during labour. There were five mothers who were treated for chorioamnionitis in the group.

Infant characteristics

All infants (*N*=77) were inborn. They had a median (IQR) weight of 1 000 (900 -1 120) g and a median (IQR) gestation of 30 (28 - 31) weeks. There were 39 male infants. One infant was transferred to a tertiary institution owing to suspected ileal atresia, and he returned after surgery. All infants received exclusive EBM and/or *d*EBM until they weighed 1 500 g. Probiotics were administered to all infants within 24 hours, and were stopped when the infants reached 1200 g or soon afterwards.

All 13 RVD-exposed infants' birth polymerase chain reaction (PCR) tests were negative. The 10-week PCR results were negative in 11 (84.6%) infants and not done in 2 (15.4%).

Outcomes

A total of 11 (14.3%) infants had positive cultures. One of the infants had 2 positive cultures on separate days and one infant grew 2 different organisms on the same culture. (Table 1). There were no positive cultures of probiotic organisms. Nine (81.8%) of the infants who had positive cultures were ELBW infants.

There was a total of seven (9%) deaths, with 3 of those occurring within the first 72 hours of life. The other four (5%) all died of LOS. Two of these cultured *Enterobacter cloacae* and one cultured both *E. cloacae* and *Serratia marcescens*. One baby cultured *Klebsiella pneumoniae* and died on the day the positive culture was obtained. The rest of the infants died 1 - 2 weeks after their positive cultures (Table 1).

NEC was also an important factor to measure due to its potential devasting effects. No NEC was observed or diagnosed amongst this group of infants during the study period.

Comparison with previous time periods:

From January 2016 to May 2017 there were no probiotics in use. Single-strain probiotics were used from June 2017 to January 2019 and multi-strain probiotics from February 2019 to July 2020 (Fig. 1).

The number of NEC episodes during the time periods are illustrated in Fig. 2. There were no NEC cases reported after the introduction of multi-strain probiotics, which is a statistically significant decrease (p=0.009).

Discussion

In the present observational study, the introduction of multi-strain probiotics was a success. Two infants were excluded from the study as they did not meet the weight criteria. The staff, especially the nurses, were well informed about the probiotic procedures and were vigilant about initiation, daily administration and stopping the probiotics. Frequent assessment on ward rounds by the doctors ensured compliance.

As this was a small observational study with historical controls only, no conclusions can be made from the findings. However, it was interesting to note the differences between time periods in a secondary-level hospital setting.

In this group of infants 11 out of 77 (14.3%) had sepsis and 4 of those infants died. This rate was higher than that reported in a previous study (also conducted in Cape Town) but falls within the interquartile range of neonatal units in a large international database.[16] It is important to note that these comparative studies were for VLBW infants and not only the 800 - 1 200 g infants in our study. The cultures were predominately Gram-negative organisms with only 1-gram positive culture (*Enterococcus faecalis*). This correlates with a study by Dramowski *et al.*,[17] which found that sepsis is a leading neonatal cause of death in sub-Saharan Africa and is mainly caused by Gram-negative pathogens with substantial antimicrobial resistance. Four of the infants with positive cultures died. Although three of these died more than a week after the positive culture, the deaths were assumed to be related to the sepsis or a complication thereof.

When comparing the three time periods there was no significant change in the incidence of sepsis in the 800 - 1 200 g infants, nor in deaths related to sepsis. A review by Neu *et al.*[7] evaluated the effect of probiotics for LOS prevention in preterm infants according to type of feeding (EBM v. exclusive formula or mixed feeding). Overall, probiotic supplementation resulted in a significantly lower incidence of LOS only in human milk-fed preterm infants (relative risk 0.75 (95% CI 0.65 - 0.86)).

Table 1. Details of infants with confirmed sepsis

				Sepsis		
Infant	Birthweight (g)	GA (weeks)	Day of life	Episode 1	Episode 2	Outcome (day of death)
1	980	28+0	10	*Klebsiella pneumoniae, Enterobacter cloacae*	N/A	Alive
2	920	28+0	25	*K. pneumoniae*	N/A	Died (25)
3	1140	28+3	11	*K. pneumoniae*	N/A	Alive
4	980	28+6	4	*K. pneumoniae*	N/A	Alive
5	840	32+0	13	*Serratia marcescens*	N/A	Alive
6	880	28+0	12/21	*S. marcescens*	E. cloacae	Died (29)
7	1160	31+0	4	*E. cloacae*	N/A	Died (19)
8	810	28+0	7	*E. cloacae*	N/A	Died (19)
9	840	31+0	32	*E. cloacae*	N/A	Alive
10	820	31+0	8	ESBL *Klebsiella*	N/A	Alive
11	920	28+0	5	*Enterococcus faecalis*	N/A	Alive

GA = gestational age (in weeks^{+days}); N/A = not applicable; ESBL *Klebsiella* = extended-spectrum β-lactamase-producing *Klebsiella pneuomoniae* (ESBL-*Kp*).

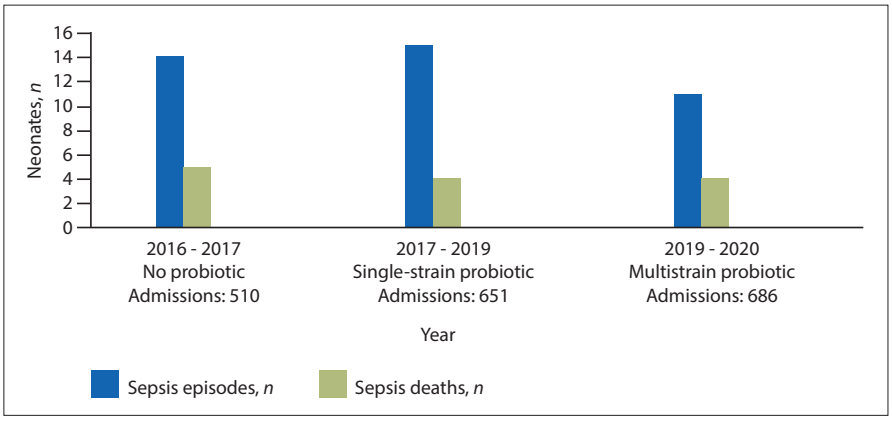

Fig. 1. Sepsis episodes and deaths during the three time periods. Admissions are the total number of all infants admitted to the unit during the time periods. The differences in sepsis rates (2.7%, 2.3% and 1.6%) were not statistically significant (p=0.39).

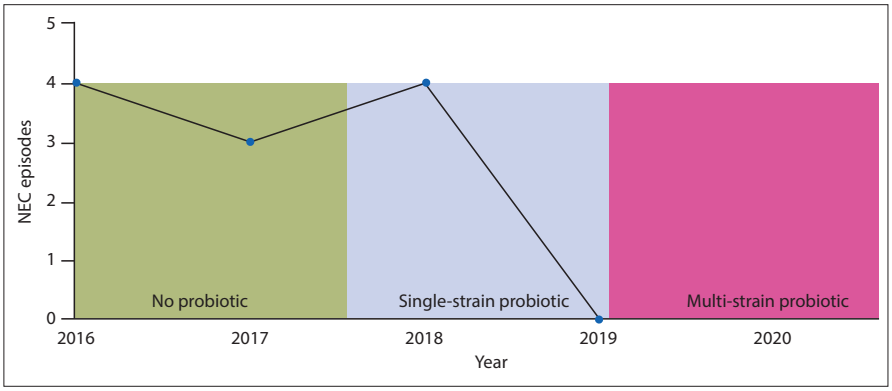

Fig. 2. NEC episodes in the 800 - 1 200 g infants during the no probiotic, single strain and multi-strain probiotic time periods.

However, the present study illustrated a decrease in the incidence of NEC, as there were no cases during the study period. Compared with previous time periods, the rate of NEC was less when single-strain probiotics were administrated compared with when no probiotics were used. There is strong evidence that probiotics decrease NEC rates in VLBW infants. A meta-analysis by Deshpande *et al.*[18] reported significant benefits of probiotic supplementation in reducing death and the risk of NEC in preterm neonates. However, many questions remain. Robertson *et al.*[12] showed that although probiotics may reduce the incidence of NEC in preterm infants less than 28 weeks' GA, the evidence was still low for ELBW (<1 000 g) neonates. This is especially important in this study where half of the infants in the study were ELBW.

Although invasive sepsis from probiotic organisms is very rare, it can be devasting, as seen recently in the USA, where the Food and Drug Administration released a new warning raising concerns about the use of probiotics, following the death of an ELBW infant in October 2023.[19]

If the controversies around probiotics are resolved, for example the cost, strain preferences and more research about ELBW infants, they would be a welcome addition to the management of preterm infants. NEC has a high mortality and morbidity in SA and often requires the use of TPN and prolonged antibiotic use.[20]

Other factors which may have contributed to the decrease in NEC included a strict breastmilk policy for all infants <1 500 g, standardised feeding protocols and a strict hygiene policy.

The cost-effectiveness of probiotics, especially in low-income countries with resource limitations, has been investigated. In 2020 Atoosa *et al.*[21] examined the cost-effectiveness of prophylactic probiotics on NEC prevention in VLBW infants and concluded that prophylactic probiotics were a cost-effective strategy in NEC reduction. The hospital used two vials of probiotics per month (ZAR842 per vial in 2020) – the cost was not prohibitive. However, the cost saving on vial sharing should always be weighed against the potential of cross-infection should the vials become infected with pathogenic bacteria. This protocol also halted the administration of probiotics once the infants reached 1 200 g, which means some infants received many more doses than others. To the best of our knowledge, there are no studies on the relationship between colonisation by probiotic organisms and duration of probiotic administration in preterm infants.

Study limitations

The present study included a small retrospective sample of infants with only a historical control group and no placebo arm and was therefore purely observational. We were also unable to ascertain the exact number of admissions during each time period in the 800 - 1 200 g category and therefore used the total admissions of all infants during those periods to calculate sepsis rates. Although this was not ideal, the ratio of 800 - 1 200 g infants is likely to be relatively similar between time periods. Changes in unit policy over time may affect outcomes. However, the same neonatologist was in charge for the entire study period and there were no major changes to unit protocols.

Conclusion

It is possible to effectively introduce probiotics at a secondary-level hospital neonatal nursery. The present observational study showed a similar rate of LOS when compared with other time periods and there were no cases of NEC.

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