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Original Article

Impact of a Multi-Strain Probiotic on Healthcare-Associated Bloodstream Infection Incidence and Severity in Preterm Neonates

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ABSTRACT

Aim: Hospital acquired bloodstream infection (HA-BSI) is a major contributor to morbidity and mortality in preterm, very low birthweight infants, especially in low-to-middle- income countries.

Materials and Methods: We conducted a double-blind, placebo-controlled, randomized clinical trial to investigate the effect of a multi-strain probiotic formulation (LabinicTM) on the incidence and severity of HA-BSI in preterm neonates.

Results: Two hundred neonates (100 per arm) were included in this trial. Fifteen neonates developed HA-BSI events (2 in the probiotic arm and 13 in the placebo arm). The median day of life at HA-BSI onset for the probiotic group was 10.5±3.5, and for the placebo group, it was 11.2±6.4. The incidence of HA-BSI in neonates receiving the probiotic was significantly lower compared to those receiving the placebo [0.93 versus 5.99 HA-BSI events/1,000 neonate-days; incidence rate ratio (IRR) of 0.156 [95% confidence interval (CI): 0.017 to 0.691], p=0.0046]. Calculating the incidence rate of the combined outcome (sepsis/death) was also lower in the probiotic group versus the placebo group [2.34 versus 6.45 events/1,000 neonate days; IRR 0.33 (95% CI: 0.11 to 0.97), p=0.043].

Conclusion: The use of a multi-strain probiotic significantly reduced HA-BSI incidence in this cohort of preterm neonates.

Keywords: Healthcare-associated bloodstream infection, neonate, probiotic

Introduction

Globally, neonatal infections cause an estimated 26% of all neonatal deaths, with the highest infection-related mortality observed in Sub-Saharan Africa (1,2). Hospital acquired bloodstream infection (HA-BSI), defined as BSI occurring 48-72 hours after birth, are the most frequent infection type encountered in hospitalised neonates (3). The incidence of HA-BSI is inversely related to neonatal gestational age and birth weight with preterm (<37 weeks gestation) and very low birth weight neonates (<1,500 g) at particularly elevated risk (4).

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Dong and Speer (3) analysed data from 11 studies globally concluding that extremely preterm (<28 weeks' gestation) versus late preterm neonates (33-36 weeks' gestation) had a two-fold higher HA-BSI prevalence (36% vs 18%). In South African hospitals, approximately 1 in 10 preterm neonates develop HA-BSI. Lebea (5) found an incidence rate of blood-culture confirmed neonatal sepsis of 10.3 per 100 admissions, with HA-BSI accounting for 83.7% of all BSI epsiodes. Similar results were found by Motara et al. (6) with 8.1% of hospitalised neonates developing HA-BSI. In Sub-Saharan Africa, gram-negative pathogens cause two-thirds of neonatal HA-BSI and are often multidrug-resistant with substantially higher mortality rates than those observed for Gram-positive pathogens (7,8).

Neonates with a birth weight <1,500 g show delayed intestinal colonisation with normal microbial flora (3). Contributing factors include birth by caesarean section, lengthy antibiotic use, use of infant formula and parenteral nutrition, delayed skin contact and sub-optimal infection prevention practices in hospital. This abnormal gut flora (dysbiosis) can lead to bacterial translocation and HA-BSI in preterm neonates (9). An additional risk factor for HA-BSI includes the presence of invasive or indwelling devices. Important gastrointestinal risk factors for HAI include immature mucosal gut barriers, intestinal ischemia, hyperosmolar injury, bacterial invasion, and subsequent inflammation (10).

The human gut microbiome plays a pivotal role in nutritional, physiological, immunological, and protective functions (11). However, the neonatal gut microbiome may be altered, or have delayed maturation following preterm birth, antibiotic administration, and/or delays in establishing enteral feeds (12). These factors reduce the activity of beneficial bacteria e.g., *Lactobacilli* and *Bifidobacteria* and promote overgrowth of pathogenic gut bacteria, resulting in bacterial translocation and the potential to develop HA-BSI (13).

Providing probiotic therapy to preterm neonates may promote intestinal colonisation with normal, beneficial microbial flora and prevent the overgrowth of pathogens (7). The putative mechanisms of BSI prevention through probiotic supplementation include modulation of immune response parameters with increased anti-inflammatory cytokine production and stabilization of the gut barrier function, with improved intestinal integrity and colonization resistance (14,15). The effect of probiotic supplements is enhanced in neonates receiving breastmilk feeds, possibly owing to breastmilk-induced reductions in gut permeability to pathogen translocation and anti-infective components such as lactoferrin, IgA, IgG, IgM and oligosaccharides, which act synergistically as a prebiotic (8).

In a systematic review and meta-analysis, Dermyshi et al. (16) showed that probiotics reduced neonatal sepsis rates by 12% and 19% (pooled data from randomized controlled trials and observational studies, respectively). They concluded that the use of the *Lactobacillus* species or a mixture of 2-3 species of bacteria might be the most efficacious (16). Although the modest benefits of probiotics for HA-BSI prevention are promising, the optimal microbial strains, combinations, dosing, timing and duration of supplementation, and their efficacy in neonates has not been definitively elucidated. We aimed to determine whether the administration of a multi-strain probiotic could reduce the incidence and severity of HA-BSI in African neonates.

Materials and Methods

Study Design

We conducted a double-blind, placebo-controlled, randomized clinical trial to investigate the effect of a multi-strain probiotic formulation on the incidence and severity of HA-BSI in preterm neonates. This manuscript was prepared in accordance with the CONSORT statement checklist for the reporting of clinical trials.

Study Setting

Tygerberg Hospital (TBH) situated in Cape Town, South Africa, is a tertiary hospital with 1,384 beds, serving the Cape Metro Region's Northern and Eastern sub-districts and the surrounding rural districts' healthcare facilities. The neonatal unit inside TBH consists of 132 beds, including a 12-bed medical/surgical neonatal intensive care unit, 2 high-dependency wards, 1 low-care ward and 1 kangaroo mother care ward. Study participants were recruited from the two high-dependency neonatal wards. Participants were enrolled between the 19th January and 27th June, 2021.

Study Participants

Preterm neonates aged 1-3 days of life at enrolment, with a birth weight between 750-1,500 grams and <37 weeks' gestation were eligible for inclusion. Exclusion criteria were severe or life-threatening congenital anomalies, early onset neonatal sepsis [C-reactive protein (CRP) >10 mg/L in the first 72 hours of life], (17) neonates scheduled for adoption, major gastro-intestinal abnormalities, or surgery of the gastro-intestinal tract. This study had four main outcomes, of which HA-BSI compromised one. We used one of the

other study outcomes, namely a reduction in the carriage rate of antibiotic resistant organisms, to calculate the required sample size for this study. A proportion difference of a 17% decrease in rectal colonisation with drug-resistant bacteria was used to estimate the sample size required to detect a significant difference between the groups being compared (with a Type I error of 0.05 and a power of 80%). The total sample size required was 200 neonates or 100 per group (allowing for a 12% margin for study participants lost-to-follow-up).

Randomisation

A pre-determined randomization list prepared by the study statistician was used to randomly allocate neonates to the two balanced study arms (n=100 each) - a probiotic (intervention) group and a placebo group. Consecutive sampling was used i.e., every preterm neonate meeting the inclusion criteria was selected until the required sample size was achieved.

The manufacturer packaged the products (probiotic or placebo) and did the allocation concealment. The packaging of the two products was identical apart from a distinguishing pink or green sticker. Once enrolled, each neonate received their own probiotic or placebo bottle in order to avoid contamination and to ensure that the infant received the same treatment over time. The researcher and all neonatology staff were blinded as to which of the two groups received the probiotic versus the placebo.

Procedures

A multi-strain probiotic containing *Lactobacillus acidophilus* [0.67 billion colony forming units (CFU)s], *Bifidobacterium bifidum* (0.67 CFUs) and *Bifidobacterium infantis* (0.67 CFUs) was used, Labinic™ (Biofloratech, Surrey, United Kingdom). The placebo consisted of medium chain triglyceride oil and Aerosil 200 (Aerosil 200 is the stabiliser also used in Labinic™).

The standard dose of 0.2 mL was administered, providing 2 billion CFUs per day. Supplementation with the probiotic or placebo was delayed if the neonate was *nil per os* and discontinued if a neonate developed necrotizing enterocolitis (NEC) (Bells stage II or more) (18). The researcher added the probiotic/placebo to the neonate's feed (mother's own breast milk/donor breast milk/neonate formula) before administration of the feed via an orogastric tube or if applicable, orally. The probiotic/placebo was administered once daily to the neonate's morning feed and the neonates were followed up from birth to a maximum of 28 days of life, death, discharge to peripheral hospitals or home, depending on whichever time-point came first.

Data collected at enrolment included neonatal demographic information, estimated gestational age (early/late ultrasound or foetal foot length), gender, birth weight, type of delivery, ethnicity and Apgar scores. Daily data collected included reviewing the clinical notes, laboratory records, anthropometric measurements, recording the type and volume of feeds received, infections present (e.g., meningitis, urinary tract infection, pneumonia, tuberculosis) and any medication prescribed.

HA-BSI was defined as a positive blood culture with a known neonatal pathogen obtained after 72 hours of life together with a CRP above 10 mg/L (19). HA-BSI was excluded in the presence of a negative blood-culture and/or a CRP<10 mg/L. Central line associated bloodstream infections are not part of HA-BSI and were not part of the protocol. Organisms were classified using the United States Centers for Disease Control list of pathogens and contaminants (https://www.cdc.gov/hai/organisms/organisms.html). Repeat blood cultures isolating the same pathogen within 10 days of the original specimen were considered to represent a single episode of infection. VLBW infants with blood cultures isolating known skin commensals or contaminants were excluded from further study end point analysis (20). A poly-microbial infection was defined as the isolation of more than one pathogenic organism from a single blood culture.

Hospital guidelines recommend routine blood culture collection at birth for neonates with obstetric risk factors for infection e.g., prolonged rupture of membranes, chorioamnionitis, or suspected sepsis. Neonates who develop clinical signs and symptoms of infection during hospital admission also undergo a sepsis work-up including full blood count, CRP and blood culture collection as minimum laboratory investigations. Approximately 1-2 mL aseptically collected blood is inoculated into a paediatric blood culture bottle (BacT/ALERT PF bottle) and submitted to the on-site National Health Laboratory Services (NHLS) which uses the automated BacT/Alert blood culture system (BioMerieux, Marcy l'Etoile, France). If bacterial growth is detected, a Gram stain is performed and the sample sub-cultured onto appropriate media and incubated overnight. Further identification and antimicrobial susceptibility testing of clinically significant isolates is performed with the automated Vitek II system (BioMerieux) using Clinical and Laboratory Standards Institute breakpoints. If urinary

tract infection, meningitis or another infection focus is suspected, additional laboratory specimens are submitted.

In most instances, the following antibiotics are used (local hospital guidelines): ampicillin and gentamicin if the neonate is <72 hours of life; if the neonate is ≥72 hours of life, piperacillin-tazobactam plus amikacin is used for stable neonates, and meropenem for critically ill neonates or neonates with suspected meningitis. Neonates with HA-BSI in the presence of thrombophlebitis or the recent use of central lines have vancomycin added to their antibiotic treatment at the clinician's discretion. Following pathogen identification and antibiotic susceptibility testing, the empiric antibiotic regimen is adapted to provide the narrowest spectrum treatment possible or discontinued if the blood culture is negative.

Statistical Analysis

A baseline table of demographic and clinical characteristics were tabulated by group and contains frequencies, percentages, and medians. An intention to treat analysis comparing the probiotic vs placebo arms for HA-BSI incidence was performed. The HA-BSI incidence rates, calculated using the total number HA-BSI events in each trial arm divided by the respective neonate days x 1,000, were used to calculate the incidence rate ratio (IRR) with 95% confidence intervals (CI). A sensitivity analysis was conducted using a Poisson regression model to estimate the IRR adjusted for some baseline factors: gender, maternal age, birthweight, gestational age, and day of commencement of enteral feeds. The proportion of neonates receiving antibiotics in each group were compared using a chi-squared test. For all statistical tests performed, a p-value <0.05 was considered significant. All the statistical analyses were performed using STATA 16.0 (College Station, Texas 77845 USA).

Ethical Approval

Ethical approval was granted by the Health Research Ethics Committee of the Faculty of Health Sciences of Stellenbosch University as well as Tygerberg Hospital (S20/07/178). This trial was registered in the Pan African Clinical Trial Registry (PACTR202011513390736). Written informed consent was obtained from each neonate's mother.

Role of the Funding Source

The funders of this trial had no role in trial design, data collection, data analysis, data interpretation, or the writing of this report.

Results

A total of 709 neonates were screened for this study, of which 313 were eligible for inclusion. Of these neonates, 207 were enrolled, but 7 neonates developed early complications prior to receiving the placebo/probiotic and they were excluded from subsequent analysis. Two hundred neonates (100 per arm) were included in this trial (Figure 1). Of the 200 enrolled neonates, 100 (50%) completed the full 28-day study period in the neonatal unit and the remainder were either transferred to other hospitals (47%), discharged (0.5%) or passed away (2.5%). The mean number of days enrolled in this study was similar between the two groups: probiotic 21.35 (±7.69) days and placebo 21.70 (±7.62) days.

The participants' mean gestational age was 29 weeks ±13.9 days (range 25-36 weeks), in the probiotic group and 30 weeks ±13.5 days (range 25-34 weeks) in the placebo group. The participants' mean birth weight was 1,174 g ±226 g (range 780 g-1,500 g) in the probiotic group and 1,150 g ±230 g (range 750 g-1,495 g) in the placebo group. Nearly a quarter of the neonates (23%) were HIV-exposed, but none returned a positive HIV PCR test at birth (Table I). The mode of delivery did not differ between the two groups and nearly three out of four neonates (73%) were delivered by caesarean section (Table II).

The mean day of life at HA-BSI onset for the probiotic group was 10.5±3.54, (range 8-10 days) and for the placebo

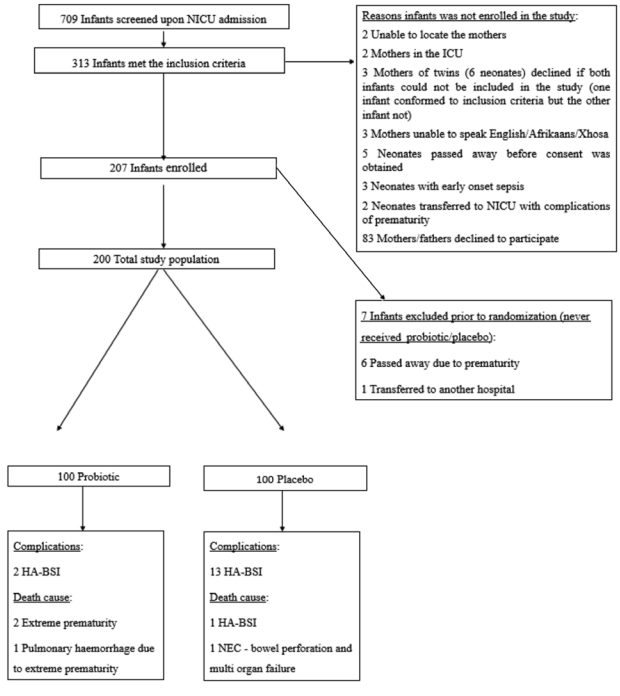

Figure 1. Flow diagram of neonates included in the clinical trial

Table I. Demographic and clinical characteristics of neonates enrolled in the study (n=200)

	Probiotic group (n=100)	Placebo group (n=100)
Gender		
Male (n, %)	47 (47)	37 (37)
Female (n, %)	53 (53)	63 (63)
Birth weight		
750-1000 g (n, %)	30 (30)	32 (32)
1001-1500 g (n, %)	70 (70)	68 (68)
Gestational age		
26-28 weeks (n, %)	34 (34)	30 (30)
29-32 weeks (n, %)	60 (60)	62 (62)
33-36 weeks (n, %)	6 (6)	8 (8)
Apgar score (10 min)		
<4 (n, %)	0 (0)	1 (1)
4-7 (n, %)	10 (10)	9 (9)
>7 (n, %)	89 (89)	89 (89)
No Apgar (born before arrival (n, %)	1 (1)	1 (1)
HIV		
Exposed (n, %)	22 (22)	26 (26)
Unexposed (n, %)	78 (78)	74 (74)
First feed received		
EBM (n, %)	68 (68)	69 (69)
DEBM (n, %)	12 (12)	6 (6)
PEBM (n, %)	19 (19)	25 (25)
FM (n, %)	1 (1)	0 (0)
Subsequent feeds received*		
EBM (n, %)	63 (63)	66 (66)
DEBM (n, %)	13 (13)	9 (9)
PEBM (n, %)	15 (15)	24 (24)
FM (n, %)	9 (9)	1 (1)

*The feed received most often (>50% of the time)
DEBM: Donor expressed breastmilk, EBM: Expressed breastmilk, FM: Formula milk, PEBM: Pasteurized expressed breastmilk

group, it was 11.15±6.37, (range 4-28 days). The incidence of HA-BSI in the probiotic arm was significantly lower compared to those receiving the placebo (Table III). HA-BSI episodes occurred in 2 (2%) neonates receiving the probiotic and in 13 (13%) neonates in the placebo group. The incidence of HA-BSI in neonates receiving the probiotic was significantly lower compared to those receiving the placebo [0.93 versus 5.99 HA-BSI events/1,000 neonate-days; IRR of 0.156 (95% CI: 0.017 to 0.691), p=0.0046], using total neonatal study days as the denominator (2,135 days in the probiotic group and 2,170 days in the placebo group). *Klebsiella pneumoniae* was cultured in 2/2 (100%) of the neonates in the probiotic group. The organisms cultured in the placebo group varied, with the main organisms being *Serratia marcescens* 4/15 (31%), *Klebsiella pneumoniae* 3/15 (23%) and *Enterococcus faecalis* 3/15 (23%) (Table IV).

Table II. Delivery information of the mothers (n=200)

	Probiotic group (n=100)	Placebo group (n=100)
Maternal age		
18-20 years (n, %)	16 (16)	13 (13)
21-30 years (n, %)	41 (41)	52 (52)
31-40 years (n, %)	39 (39)	32 (32)
41-45 years (n, %)	4 (4)	3 (3)
Mode of delivery		
C-section (n, %)	73 (73)	73 (73)
Vaginal delivery (n, %)	27 (27)	27 (27)
Maternal steroids		
Received (n, %)	84 (84)	89 (89)
Birth number		
Single neonate (n, %)	79 (79)	86 (86)
Twin neonates (n, %)	21 (21)	14 (14)
Reason for premature delivery		
SPPROM (n, %)	16 (16)	20 (20)
FD (n, %)	57 (57)	43 (43)
EOPET (n, %)	2 (2)	4 (4)
Placenta abruption (n, %)	2 (2)	7 (7)
IUGR (n, %)	1 (1)	6 (6)
SPTL (n, %)	18 (18)	18 (18)
HELLP (n, %)	2 (2)	1 (1)
Placenta praevia (n, %)	2 (2)	1 (1)

EOPET: Early onset pre-eclampsia, FD: Foetal distress, HELLP: Haemolysis, elevated liver enzymes, low platelet count, IUGR: Intrauterine growth restriction, SPPROM: Spontaneous preterm premature rupture of the membranes, SPTL: Spontaneous preterm labour

Gender, maternal age, birth weight, gestation as well as day of starting enteral feeds were not significant covariates. Adjusting for baseline covariates, the probiotic effect of preventing sepsis showed an IRR of 0.134 (95% CI: 0.028-0.642), p=0.012.

The percentage of neonates who received empiric antibiotic therapy at birth for possible infection was similar between the two groups [placebo, n=54 (54%)

Table III. Clinical data of the neonates (n=200)		
	Probiotic group (n=100)	**Placebo group (n=100)**
Invasive interventions		
Nasogastric tube inserted days (mean; ±SD)	20.9±7.8 (range 2-28)	21.4±7.8 (range 7-28)
TPN line inserted days (mean; ±SD)	0.1; ±1.00 days (range 0-8)	0.5; ±2.2 (range 0-15)
IV-line inserted days (mean; ±SD)	8.1±2.4 (range 4-21)	8.9±4.2 (range 5-28)
Mechanical ventilated days (mean; ±SD)	0	0.1±0.9 (range 0-7)
CPAP days (mean; ±SD)	5.2±4.9 (range 0-26)	5.5±5.2 (range 0-28)
High flow days (mean; ±SD)	3.3±4.9 (range 0-21)	3.7±4.0 (range 0-20)
Nasal prongs days (mean; ±SD)	3.8±5.1 days (range 0-22)	4.3±5.0 (range 0-24)
Day on which feeds was initiated		
DOL (mean days; ± SD)	3.1±1.1 (range 0-6)	3.0±1.0, (range 2-6)
Days to achieve full feeds		
DOL (mean days; ±SD)	8.7 ±2.0 (range 5-18 days)	9.7±4.3, (range 6-28 days)
Number of days on TPN (mean days, +- SD)	0.1 days; ±1.0 (range 0-8)	0.5 days; ±2.2 (range 0-15)
Number of days NPO (mean days, +- SD)	0.2 days; ±0.4 (range 0-8)	0.4 days; ±0.7 (range 0-8)
Neonates classified as at septic risk at birth and received empiric antibiotics	57	55
Empiric antibiotic use for presumed sepsis at birth (n) Days (mean days; ±SD)	57 3.8; ±2.1, (range 1-12)	55 3.8; ±2.0, (range 1-10)
Empiric antibiotic regimens (<72 hours of life) (n; %) ampicillin plus gentamicin piperacillin-tazobactam plus amikacin vancomycin meropenem	 52 (91) 12 (21) 1 (2) 1 (2)	 53 (96) 5 (9) 0 (0) 3 (6)
Positive cultures		
Number of blood cultures submitted	89	119
Neonates with positive blood culture (n, %)	2 (2)	13 (13)
Total number of pathogens isolated from the cultures requested as per above (n=23)	2	16
Monomicrobial BSI	2	10
Polymicrobial BSI	0	3
Day of life at HA-BSI onset, (mean±SD)	10.5±3.5, (range 8-10)	11.2±6.4, (range 4-28)
Targeted antibiotic regimens used for HA-BSI episodes after blood culture results were available	n=2	n=13
Neonates that received the antibiotic (n; %) piperacillin-tazobactam plus amikacin meropenem plus vancomycin ampicillin plus gentamicin meropenem plus colistin meropenem	 0 (0) 0 (0) 0 (0) 0 (0) 2 (100)	 4 (26.5) 2 (13) 3 (23) 3 (23) 3 (23)
Infants that developed HA-BSI	n=2	n=13
Weight		
750-1000 g (n, %)	2 (2%)	8 (8%)
1001-1500 g (n, %)	0 (0%)	5 (5%)
Gestational age		
26-28 weeks (n, %)	0 (0%)	8 (8%)
29-32 weeks (n, %)	2 (2%)	5 (5%)
33-36 weeks (n, %)	0 (0%)	0 (0%)

CPAP: Continuous positive airway pressure, DOL: Day of life, NPO: Nul per os, TPN: Total parenteral nutrition, HA-BSI: Hospital acquired bloodstream infection, SD: Standard deviation

versus probiotic, n=57 (57%)]. When analysing the subset of neonates who received empiric antibiotics and later developed HA-BSI, there was no significant difference in the occurrence of sepsis between the two groups, with sepsis occurring in 3.5% of the probiotic group (2/57) neonates, and 7.3% (4/55 neonates) in the placebo group (IRR: 0.467, p=0.4064). The probability of empiric antibiotic use in the probiotic group was 1.09 times as high as for the placebo group but not significantly different (p=0.788).

HA-BSI incidence rates were higher among neonates who did not received antibiotics at birth, compared to those who had (9/88 vs 6/112; p=0.194). In the sub-group considered not at risk of sepsis at birth, with no empiric antibiotic use, 0% (0/43) of these infants developed sepsis in the probiotic group, versus 20% (9/45) in the placebo group (p<0.004).

Five neonates passed away, 2 in the placebo group passed away (HA-BSI on day 8 of life, NEC on day 21 of life) and 3 in the probiotic group (2 from extreme prematurity on day 7 of life, and one from pulmonary haemorrhage on day 15 of life). There was a significant risk reduction in survival for neonates in the probiotic group. The incidence rate of the combined outcome (sepsis/death) was lower in the probiotic group versus the placebo group [2.34 versus 6.45 events/1,000 neonate days; IRR 0.33 (95% CI: 0.11 to 0.97), p=0.043].

In calculating the sepsis/death incidence rate per 1,000 neonate-days, there were 2.34 events in the probiotic group versus 6.45 in the placebo group. The IRR of probiotic relative to placebo sepsis/death events was 0.33 (95% CI: 0.11 to 0.97), p=0.043.

Other infection types that were documented during the trial included: urinary tract infection (1, placebo group), congenital tuberculosis (1, probiotic group), and pneumonia (3 in the probiotic and 1 in the placebo group).

No protocol violations nor serious adverse events relating to the use of the probiotic occurred.

Table IV. Neonatal HA-BSI pathogens

	Probiotic group (n=2) n (%)	**Placebo group (n=16*) n (%)**
Organisms isolated		
Klebsiella pneumoniae	2 (100%)	3 (19%)
Serratia marcescens	0 (0%)	4 (25%)
Enterococcus faecalis	0 (0%)	3 (19%)
Staphylococcus aureus	0 (0%)	2 (12.5%)
Acinetobacter baumannii	0 (0%)	2 (12.5%)
Klebsiella oxytoca	0 (0%)	1 (6%)
Proteus mirabilis	0 (0%)	1 (6%)

HA-BSI: Hospital acquired bloodstream infection
*(10 Infants had a monomicrobial BSI, and 2 infants had a polymicrobial BSI).

Discussion

HA-BSI is a leading cause of illness and death in hospitalised preterm neonates in South Africa (5,6,21). South African data shows that around 10% of preterm neonates develop HAI (5). In our study, the probiotic group showed an 84% risk reduction in the incidence of HA-BSI, compared to the placebo group when a multi-strain probiotic, Labinic™, was administered daily for a duration of up to 28 days. A review of previous probiotic studies confirms that multi-strain probiotics are preferable to single-strain probiotics, as they were more likely to be associated with a statistically significant reduction in HA-BSI rates and/or death. A systematic review and meta-analysis by Dermyshi et al. (16) in 2017 recommended that a multi-strain probiotic containing *Lactobacillus acidophilus* together with *Bifidobacterium infantis* or others should be considered. Their analysis showed that single-strain probiotics e.g., *Lactobacillus reuteri*, *Bifidobacterium breve* or *Saccharomyces boulardii,* had no effect in reducing HA-BSI or mortality (16). Kanic et al. (22) also showed a statistically significant reduction in HAI when using a multi-strain probiotic containing *Lactobacillus acidophilus* (*subsp. Lactobacillus Gasseri), Bifidobacterium infantis* and *Enterococcus faecium*. Unfortunately, a large prospective trial (the Proprem trial) using *Bifidobacterium lactis, Streptococcus thermophilus* and *Bifidobacterium infantis* showed no significant reduction in sepsis or mortality (23). At the same time, most single-strain trials failed to show any beneficial effects. A randomized controlled trial by Mihatsch et al. (24) showed that a single strain of *Bifidobacterium lactis* did not reduce the incidence of nosocomial infections in VLBW infants. A multicentre trial by Dani et al. (25) using *Lactobacillus rhamnosis* also showed no significant reduction in bacterial sepsis compared to a placebo.

The diagnosis of HAI may be difficult to confirm and thus empiric antibiotic therapy is promptly initiated for neonates at high-risk of infection e.g., prolonged rupture of membranes, or chorioamnionitis (26). The use of empiric antibiotics in our study was similar between the probiotic and placebo groups (3.75 days vs 3.80 days). However, the use of antibiotics for a confirmed HA-BSI differed between the probiotic and placebo groups (8.40 days vs 11.64 days).

In the subgroup of neonates not classified as at septic risk with no empiric antibiotic use, there was a large difference in the occurrence of HA-BSI with 0% detected in the probiotic group (0/43) vs 20% (9/45) in the placebo group ($p<0.004$). The probiotic intervention thus especially protected those neonates who did not receive empiric antibiotics.

Klebsiella pneumoniae was identified in the blood culture of both neonates who developed HA-BSI in the probiotic group. The main organisms identified in the placebo group were *Serratia marcescens*, *Klebsiella pneumoniae* and *Enterococcus faecalis.* A previous study by Dramowski et al. (27) at the same institute identified *Klebsiella pneumoniae* and *Staphylococcus aureus* as the leading neonatal pathogens. In keeping with other studies on neonatal HAI, *Serratia marcescens* was a major contributor to HA-BSI events in our trial cohort (28,29).

A limitation of this study was the high proportion of the study population who were transferred out to peripheral hospitals, owing to high occupancy rates at the tertiary hospital, which led to reduced days of observation during this trial. The NHLS database was screened for all study participants who were transferred to peripheral hospitals for subsequent blood cultures. None of the infants yielded a positive blood culture up until day 28 of life.

Besides the morbidity and mortality associated with HA-BSI, it has been shown that inflammation can also contribute to long-term neuro developmental impairment as it adversely affects the preterm brain (30).

The use of a multi-strain probiotic shows great potential as a cost effective and safe method of reducing HA-BSI and subsequent mortality in preterm neonates. Probiotics are potentially the most cost-effective intervention for the prevention of HA-BSI. As Athalye-Jape and Patole (31) concluded, no intervention comes close to probiotics in the reduction of length of stay at a cost of less than a dollar per day. Multi-strain probiotics (through reduction in HA-BSI events) could potentially reduce the length of hospital stay in preterm neonates and thus be a resource and cost saving intervention. This study showed that a multi-strain probiotic (*Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Bifidobacterium infantis)* has the potential to reduce HA-BSI, morbidity as well as mortality.

Conclusion

As medical interventions advance, and extremely preterm neonates survive in greater numbers, the incidence of HA-BSI increases. Probiotics could play an important role in preserving gut integrity and preventing severe infections in preterm neonates. In this RCT, a multi-strain probiotic containing *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Bifidobacterium infantis* given daily to preterm neonates significantly reduced the incidence of HA-BSI.

Ethics

Ethics Committee Approval: Ethical approval was granted by the Health Research Ethics Committee of the Faculty of Health Sciences of Stellenbosch University as well as Tygerberg Hospital (S20/07/178).

Informed Consent: Written informed consent was obtained from each neonate's mother.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: M.S., M.M.V.W., A.N.H.B., A.D., E.V.N., Design: M.S., M.M.V.W., A.N.H.B., A.D., E.V.N., Analysis or Interpretation: C.L., Literature Search: M.S., M.M.V.W., A.N.H.B., A.D., E.V.N., Writing: M.S., M.M.V.W., A.N.H.B., A.D., C.L., E.V.N.

Conflict of Interest: The authors declared that there were no conflicts of interest.

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References

1. Lawn JE, Cousens S, Zupan J; Lancet Neonatal Survival Steering Team. 4 million neonatal deaths: when? Where? Why? Lancet 2005; 365:891-900.
2. Ranjeva SL, Warf BC, Schiff SJ. Economic burden of neonatal sepsis in sub-Saharan Africa. BMJ Glob Health 2018; 3:e000347.
3. Cortese F, Scicchitano P, Gesualdo M, et al. Early and Late Infections in Newborns: Where Do We Stand? A Review. Pediatr Neonatol 2016; 57:265-73.
4. Dong Y, Speer CP. Late-onset neonatal sepsis: recent developments. Arch Dis Child Fetal Neonatal Ed 2015; 100:F257-63.
5. Lebea MM. Evaluation of culture-proven neonatal sepsis at a tertiary care hospital in South Africa (dissertation). University of the Witwatersrand, 2015. http://wiredspace.wits.ac.za/handle/10539/19970 (accessed 12 August 2016).
6. Motara F, Ballot DE, Perovic O. Epidemiology of neonatal sepsis at Johannesburg Hospital. South Afr J Epidemiol Infect 2005; 20:90-3.
7. Olsen R, Greisen G, Schrøder M, Brok J. Prophylactic Probiotics for Preterm Infants: A Systematic Review and Meta-Analysis of Observational Studies. Neonatology 2016; 109:105-12.
8. Zhang GQ, Hu HJ, Liu CY, Shakya S, Li ZY. Probiotics for Preventing Late-Onset Sepsis in Preterm Neonates: A PRISMA-Compliant Systematic Review and Meta-Analysis of Randomized Controlled Trials. Medicine (Baltimore) 2016; 95:e2581.

9. Gareau MG, Sherman PM, Walker WA. Probiotics and the gut microbiota in intestinal health and disease. Nat Rev Gastroenterol Hepatol 2010; 7:503-14.

10. Gregory KE, Deforge CE, Natale KM, Phillips M, Van Marter LJ. Necrotizing enterocolitis in the premature infant: neonatal nursing assessment, disease pathogenesis, and clinical presentation. Adv Neonatal Care 2011; 11:155-64; quiz 165-6.

11. Hooper LV, Midtvedt T, Gordon JI. How host-microbial interactions shape the nutrient environment of the mammalian intestine. Annu Rev Nutr 2002; 22:283-307.

12. Cong X, Xu W, Janton S, et al. Gut Microbiome Developmental Patterns in Early Life of Preterm Infants: Impacts of Feeding and Gender. PLoS One 2016; 11:e0152751.

13. Totsu S, Yamasaki C, Terahara M, Uchiyama A, Kusuda S; Probiotics Study Group in Japan. Bifidobacterium and enteral feeding in preterm infants: cluster-randomized trial. Pediatr Int 2014; 56:714-9.

14. Wolvers D, Antoine JM, Myllyluoma E, Schrezenmeir J, Szajewska H, Rijkers GT. Guidance for substantiating the evidence for beneficial effects of probiotics: prevention and management of infections by probiotics. J Nutr 2010; 140:698S-712S.

15. Millar M, Wilks M, Costeloe K. Probiotics for preterm infants? Arch Dis Child Fetal Neonatal Ed 2003; 88:F354-8.

16. Dermyshi E, Wang Y, Yan C, et al. The "Golden Age" of Probiotics: A Systematic Review and Meta-Analysis of Randomized and Observational Studies in Preterm Infants. Neonatology 2017; 112:9-23.

17. Stoll BJ, Hansen N, Fanaroff AA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. Pediatrics 2002; 110(2 Pt 1):285-91.

18. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. Pediatr Clin North Am 1986; 33:179-201.

19. Brown JVE, Meader N, Cleminson J, McGuire W. C-reactive protein for diagnosing late-onset infection in newborn infants. Cochrane Database Syst Rev 2019; 1:CD012126.

20. Wirtschafter DD, Padilla G, Suh O, Wan K, Trupp D, Fayard EE. Antibiotic use for presumed neonatally acquired infections far exceeds that for central line-associated blood stream infections: an exploratory critique. J Perinatol 2011; 31:514-8.

21. Coetzee M, Nbowane NT, de Witt TW. Neonatal sepsis: Highlighting the principles of diagnosis and management. South African Journal of Child Health 2017; 11:99-103.

22. Kanic Z, Micetic Turk D, Burja S, Kanic V, Dinevski D. Influence of a combination of probiotics on bacterial infections in very low birthweight newborns. Wien Klin Wochenschr 2015; 127 Suppl 5:S210-5.

23. Jacobs SE, Tobin JM, Opie GF, et al. Probiotic effects on late-onset sepsis in very preterm infants: a randomized controlled trial. Pediatrics 2013; 132:1055-62.

24. Mihatsch WA, Vossbeck S, Eikmanns B, Hoegel J, Pohlandt F. Effect of Bifidobacterium lactis on the incidence of nosocomial infections in very-low-birth-weight infants: a randomized controlled trial. Neonatology 2010; 98:156-63.

25. Dani C, Biadaioli R, Bertini G, Martelli E, Rubaltelli FF. Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. A prospective double-blind study. Biol Neonate 2002; 82:103-8.

26. Ballot DE, Nana T, Sriruttan C, Cooper PA. Bacterial bloodstream infections in neonates in a developing country. ISRN Pediatr 2012; 2012:508512.

27. Dramowski A, Madide A, Bekker A. Neonatal nosocomial bloodstream infections at a referral hospital in a middle-income country: burden, pathogens, antimicrobial resistance and mortality. Paediatr Int Child Health 2015; 35:265-72.

28. Couto RC, Carvalho EA, Pedrosa TM, Pedroso ER, Neto MC, Biscione FM. A 10-year prospective surveillance of nosocomial infections in neonatal intensive care units. Am J Infect Control 2007; 35:183-9.

29. Zingg W, Posfay-Barbe KM, Pittet D. Healthcare-associated infections in neonates. Curr Opin Infect Dis 2008; 21:228-34.

30. Strunk T, Inder T, Wang X, Burgner D, Mallard C, Levy O. Infection-induced inflammation and cerebral injury in preterm infants. Lancet Infect Dis 2014; 14:751-62.

31. Athalye-Jape G, Patole S. Probiotics for preterm infants - time to end all controversies. Microb Biotechnol 2019; 12:249-53.