EVALUATING THE EFFECT OF PROBIOTICS ON SEVERE NECROTISING ENTEROCOLITIS IN PRETERM INFANTS BORN BEFORE 32 WEEKS GESTATION: A PROPENSITY-MATCHED POPULATION STUDY

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ABSTRACT

Background

Necrotising enterocolitis (NEC) is a leading cause of mortality and morbidity in preterm infants. Whilst there have been numerous clinical trials, there remains continued uncertainty concerning the benefits of probiotics and the different strains [1]. We aim to compare the effect of early probiotic exposure (within 14 postnatal days) versus no (or ≥14 days) probiotic exposure on odds of NEC.

Methods

We will conduct a observational, propensity score matched study using data from the UK National Neonatal Research Database (NNRD). Infants born <32 weeks gestation cared for in neonatal units in England and Wales between 01/01/2016 and 31/12/2022 will be included. We will exclude infants who died before day four of life and those with major congenital anomalies. A propensity score matched approach will be applied, matching for two critical variables gestational age and birth year epoch, and an additional 17 variables to control for background characteristics and initial severity of illness. We will compare outcomes between infants exposed and not exposed to probiotics in the first 14 days. The primary outcome will be severe NEC (confirmed surgically, by postmortem or a cause of death). Sensitivity analyses, including analysing the whole cohort are planned to assess robustness of the results.

KEY WORDS

Probiotics, preterm infants, necrotising enterocolitis, retrospective cohort studies, population study, propensity score, England, United Kingdom

INTRODUCTION

Necrotising Enterocolitis (NEC) remains one of the most important causes of morbidity and mortality of preterm infants for which there is no widely agreed reliably effective preventative intervention. While maternal breast milk has been shown to reduce the risk of NEC, the majority of NEC occur in exclusively maternal milk fed infants [2-4] . Over the past several decades the possibility that probiotics, live organisms consumed orally into the gut, might safely prevent NEC has been investigated in an extensive series of randomised controlled trials and observational studies examining different probiotic strains. The interpretation of these studies has been controversial and has resulted in a potentially confusing range of advice to clinicians.

The largest randomised controlled trials (RCT) were powered to examine the primary outcome of late-onset-sepsis, and were underpowered to examine necrotising enterocolitis [3, 4]. When individual studies were meta-analysed in a Cochrane review in 2023, probiotics were shown to reduce the risk of NEC (RR 0.54, 95% CI 0.46-0.65; I^2 = 17%; 57 trials, 10,918 infants; low certainty); probably reduce mortality (RR 0.77, 95% CI 0.66-0.90; I^2 = 0%; 54 trials, 10,484 infants; moderate certainty); and have little or no effect on the risk of late-onset invasive infection (RR 0.89, 95% CI 0.82-0.97; I^2 = 22%; 49 trials, 9876 infants; moderate certainty). Whilst data for extremely low birthweight (<1kg) infants were limited, the authors concluded that probiotics may have little or no effect on NEC (RR 0.92, 95% CI 0.69- 1.22, I^2 = 0%; 10 trials, 1836 infants; low certainty), all-cause mortality (RR 0.92, 95% CI 0.72- 1.18; I^2 = 0%; 7 trials, 1723 infants; low certainty), or late-onset invasive infection (RR 0.93, 95% CI 0.78-1.09; I^2 = 0%; 7 trials, 1533 infants; low certainty) in this group. The review recommends further large randomised controlled trials to provide evidence of sufficient validity and applicability to inform policy and practice [1].

Recommendations from professional bodies vary globally. The European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) made conditional recommendations in 2020, that certain probiotic strains and probiotic combinations could be used as potential preventative strategies for NEC [5]. In 2021 the American Academy of Pediatrics issued a statement not supporting routine universal probiotic administration, especially to infants <1000g, citing a lack of evidence of benefit in modern trials and limited availability of suitable pharmaceutical grade probiotics (in the United States) [6]. More recently, following concerns about links to cases of sepsis, the US Federal Drug Administration (FDA) has issued warning letters to companies manufacturing probiotics for illegally selling probiotic products to treat or prevent diseases in preterm infants in the absence of FDA approval for this use [7].

Probiotic use in United Kingdom neonatal units has increased significantly over the past six years, with a survey conducted in 2022 showing that around 44% (70/161) of responding units in year 2022 routinely use probiotics [8]. The two most commonly used probiotics in the UK are a combination of *Bifidobacterium infantis, Streptococcus thermophilus* and *Bifidobacterium lactis* (trade name PROPREMS abbreviated as PP) and a product containing *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *Bifidobacterium longum* spp *infantis* (trade named Labinic, abbreviated as LB) [8]. Infant-level descriptive data from England and Wales confirmed the rising use of probiotics over time such that half of infants of infants born in year 2022 born less than 32 weeks were exposed to probiotics (unpublished work).

The UK-based National Neonatal Research Database (NNRD) includes data from all infants admitted to neonatal units in England and Wales (approximately 8,000 of the 100,000 admissions per year are preterm infants born <32 weeks gestation) and provides an opportunity to assess the impact of an intervention on specific neonatal outcomes using real world data.

In this study we aim to apply causal inference methods, including propensity matching to NNRD data to determine whether probiotic use is associated with a reduction in severe NEC in infants born < 32 weeks gestation.

METHOD

Study design

This retrospective cohort study will use existing, routinely collected data held in the NNRD.

Data source

The NNRD contains records for all infants admitted to neonatal units in England and Wales since 2012 [9]. Relevant variables will be extracted from the NNRD. These included patient demographics, baseline comorbidities, diagnoses, elements of intensive care and outcomes.

Study population

Inclusion criteria: We will include infants born 23^{+0} to 31^{+6} weeks completed gestation between January 1st 2016 and December 31st 2022 (7 years) admitted to neonatal units in England and Wales.

Exclusion criteria: We will exclude infants with:

- Data missing for any of gestational age at birth, birth weight and birth year

- Data missing from the first admission to neonatal care following birth (first episode of care) or where data only exists from day 3 of life onwards
- birth weight for gestational age z score exceeds 4
- Died within the first four days of life
- Major congenital abnormality (Supplementary Material 1)

Definitions

Exposure

Exposed to probiotics: Documented receipt of any probiotic in the first 14 days of life.

Not exposed to probiotics (Comparator): No documented receipt of a probiotic in the first 14 days of life.

Primary outcome

Severe NEC defined as NEC confirmed at surgery or postmortem or NEC included as a cause of death in the NNRD [10]. (Supplementary Material 2)

Key secondary outcomes

- Alternative NEC definitions (Supplementary Material 3 for detail of how these NEC definitions will be derived from variables available in the NNRD).
    - National Neonatal Audit Programme (NNAP) definition [11] i.e. NEC diagnosed at postmortem or during surgery or using clinical and radiographic features.
    - Pragmatically defined NEC i.e. recorded diagnosis of NEC and received at least 5 consecutive days of antibiotics whilst nil by mouth.
- Late onset sepsis - Either a positive blood culture report of the growth of any organism from the NNAP late onset infection list of "Clearly pathogenic organisms" OR "other organisms" which includes coagulase negative Staphylococcus [11] OR a discharge diagnosis indicating one or more "clearly" or "other" pathogenic organisms from the NNAP lists (Supplementary Material 4).
- Survival to discharge from neonatal care

Other secondary outcomes

These include severe brain injury treated retinopathy of prematurity (ROP), bronchopulmonary dysplasia (BPD) and severe BPD (definitions in Supplementary Material 3) together with the

composite outcomes; survival without severe NEC, survival without severe NEC or late onset sepsis, survival without any NEC and time to full feeds .

Statistical analysis

Sample size and power calculation

The reduction in risk of NEC in exposed infants is of the order of 0.54 compared to those not exposed [1], and we hypothesise a similar reduction in severe NEC. The estimated UK incidence of severe NEC in this population is approximately 3.1% [2]; we therefore expect the risk in the exposed group to be 1.7%. Assuming two-sided alpha of 0.5%, matching 7,000 each of exposed and non-exposed infants would detect a difference with 99% power.

Descriptive analyses

We will report the proportion of our cohort exposed to probiotics for each year in the study period. We will also report median postnatal day when probiotics are commenced.

Missing data

We will examine rates of missingness for the exposure, outcome and all covariates. To determine whether multiple imputation is required, we will examine whether missingness of any covariate is associated with the exposure and outcome and whether the covariate value is significantly associated with the exposure and outcome.

Creating the matched sample

To address the issue of confounding, our primary analysis will use a propensity matching methodology. This will be divided into two stages; first we will estimate the probability that each infant would be exposed to probiotics (a propensity score) and second, we will match exposed infants to unexposed infants with similar propensity scores.

To create the propensity score, we first identified factors that influence probiotic receipt and risk of severe NEC by creating a directed acyclic graph (DAG) (Figure 1), informed by review of the scientific literature and pooled experience of the authors. We next mapped concepts in the DAG to related variables within the NNRD where available (Table 1). Finally, propensity scores will be created for each infant, using a logistic regression model (the propensity score model) where probiotic exposure is the dependent variable and the NNRD variables are the independent variables.

To determine which NNRD variables should be included in the propensity score model, we will assign

a level of importance (critically, highly or moderately important) to each variable. Critically and highly important variables will be mandatory in the propensity score model. The step-wise method described by Imbens and Rubins will be used to select the moderately important variables that are incorporated in the propensity score model [12] (more details in Supplementary Material 5). Critically important variables included gestational age and birth year [13]. Highly important variables include precise gestation (in days), birthweight, network, level of care on day 3 of life, sex and year of birth. Moderately important variables are shown in Table 1.

After creating the propensity scores, we will create a matched sample within strata defined by the two critically important variables, gestational age and birth year group. The groups are GA: 22+0 to 24+6 weeks, 25+0 to 27+6 weeks, 28+0 to 29+6 weeks, 30+0 to 31+6 weeks and birth year: 2016-2019 or 2020-2022, resulting in eight groups in total. Within each strata, we will match each exposed infant to an unexposed infant with a similar propensity score. We will use nearest neighbour matching with a caliper of 0.2 standard deviations. As the nearest neighbor matching method matches infants in a particular order; that order can affect the quality of both individual matches and the matched sample. We will therefore repeat the matching process twenty times, matching infants in a random order each time.

To test the association between probiotic exposure and outcomes, logistic regression will be applied to the matched sample to assess the outcomes in exposed and unexposed infants. Robust standard errors will be used to correct for correlation within a NNU and within infants born to the same mother. Bonferroni corrections will be applied to analyses of the secondary outcomes.

Mortality over the first 28 days after birth will be assessed using a log rank test and presented with Kaplan Meier curves.

Examining the incidence of NEC by probiotic strain

We will measure the association between the type of probiotic (LB or PP in the first 14 days of life) and odds of severe NEC. Given the smaller sample size of this subset of infants, we will use multivariable logistic regression rather than propensity matching. We will adjust for variables that are included in the propensity score analysis and will use robust standard errors in the logistic regression.

Whilst LB has been used in UK neonatal units since 2016, PP was only used at scale in the UK from mid 2020. To avoid confounding secular and strain influences, we will conduct a sensitivity analysis restricting the analysis to the period June 2020 to Dec 2022.

Subgroup analyses

We will conduct a pre-planned subgroup analysis for infants born < 28 weeks vs born ≥ 28 weeks to explore the treatment effect within specific populations for the primary outcome, severe NEC, and key secondary outcomes (NNAP defined NEC, pragmatically defined NEC, late onset sepsis and survival to discharge). To limit the risk of type 1 errors, we will not conduct subgroup analyses for the other secondary outcomes and Bonferroni corrections will be applied to the analyses of the secondary outcomes.

In addition, we will conduct a subgroup analysis for infants born weighing < 1kg vs ≥ 1kg. Given the substantial anticipated overlap between the populations in the gestational age subgroups and the birthweight subgroups, we will restrict this analysis to the primary outcome.

Sensitivity analyses

Whole cohort: Logistic regression without propensity matching

We will conduct a standard logistic regression analysis on the whole cohort without propensity matching to evaluate whether the findings differ. This analysis will be adjusted for all the variables included in the propensity score model for the matched sample.

Unit-level comparisons

To take account of potential cross-contamination of probiotics between infants cared for in the same NICU, we will repeat our analysis with the assumption that all infants cared for in a "*probiotic unit"* are exposed to probiotics regardless of the infant-level exposure status.

A *probiotic unit* is defined as one where:

1. Over 50% of cohort infants born in the same month, who spent day 3 of life on the unit, were exposed to probiotics in first 14 days of life OR
2. At the time the infant was born, the unit had implemented a protocol for routine use of probiotics for infants born < 32 weeks gestation. Information about protocols for probiotics was ascertained using a survey.

We will restrict this analysis to tertiary Neonatal Intensive Care Units (NICU; designation as at July 2023) because the smaller number of infants <32w in non-tertiary units may result in misclassification of a unit's probiotic status.

Because of the potential for type 1 error due to multiple comparisons, treatment effects estimated with sensitivity analyses and in subgroup analyses will be interpreted as exploratory.

Ethics

Ethics approval was granted by the South-East Scotland Research Ethics Committee 01 (REC reference 23/SS/0016) for use of the NNRD data as part of a larger study evaluating the impact of introduction of a care bundle on incidence of NEC [14].

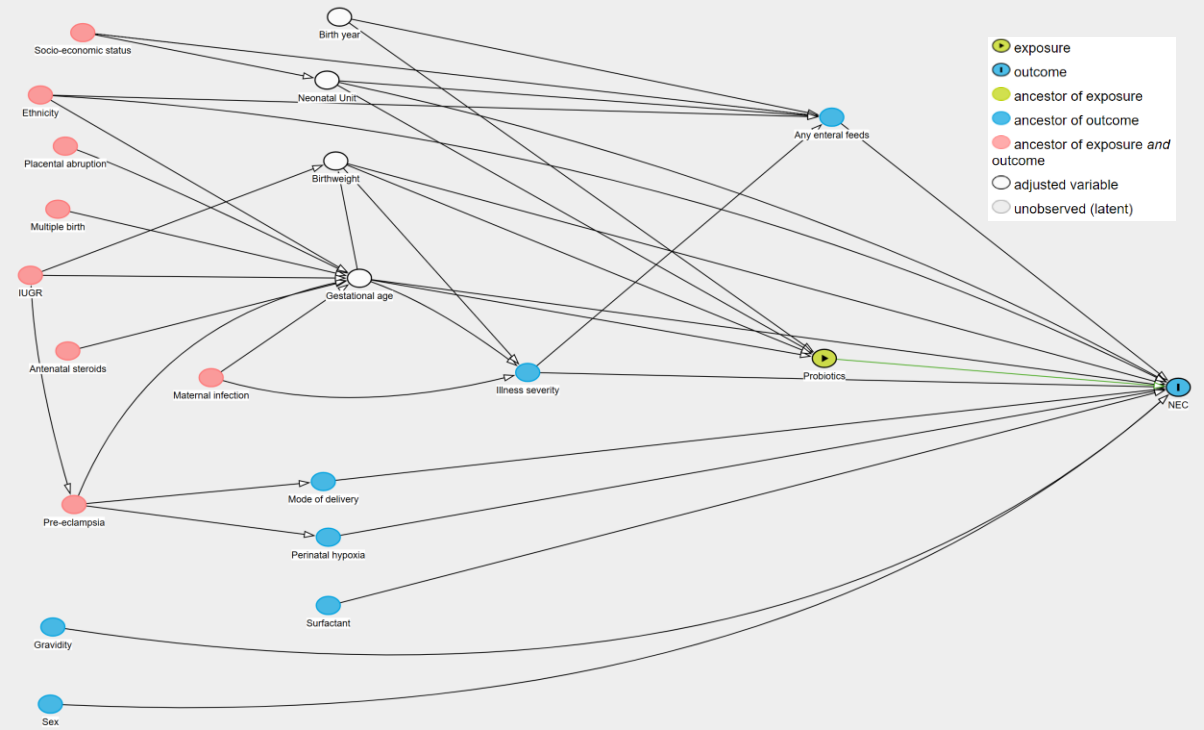
Table 1: Concepts from the directed acyclic graph and the NNRD variables which will be used to operationalise those concepts

Concept from DAG	NNRD variable used to operationalise the concept	Operationalised in propensity score
Probiotics	DrugsDay	Binary. Coded as exposed to probiotics if NNRD field DrugsDay included any one of the following terms: Labinic, Proprems, Bifidobacterium, Bio-kult, Infloran, LB2 in the first 14 days of life.
Severe necrotising enterocolitis	See Supplementary Materials 2	Binary. Coded as severe NEC present=1, No severe NEC =0
Gestational age	GestationWeeks and GestationDays	Critically important background variable. Binary <28 weeks or ≥ 28 weeks AND Highly important background variable. Continuous variable measured in days
Birth year	BirthYear	Critically important background variable. Categorical: 2016-2018, 2019-2020 or 2021-2022 AND Highly important background variable. Categorical: Measured in years
Birthweight	Birthweight	Highly important background variable. Continuous measured in grams
Neonatal Unit	ProviderNHSCode	Highly important background variable (Hospital network on day 3 after birth derived from name of neonatal unit providing care on day 3). Categorical: East Midlands; East of England; North Central & North East London; North West London; North West; Northern; South East Coast; South London; South West; Thames Valley & Wessex; Wales; West Midlands; Yorkshire & Humber.

	BAPM2011	Highly important background variable (Maximum level of care in first 4 days of life). Categorical: Intensive care, High dependency care, Special Care, Normal Care
Sex	SexPhenotype	Highly important background variable Binary: Male=1, Female=0.
Ethnicity	MumEthnicity	Moderately important background variable Categorical: White, Mixed, Asian/Asian British, Black/Black British, Other, Missing.
Index of Multiple Deprivation quintile	PostCodeMotherLSOA	Moderately important background variable (Derived from mother's Lower Level Super Output Area). Categorical: Most deprived; quintile 2; quintile 3; quintile 4; Least deprived.
Gravidity	NumberOfPreviousPregnancies	Moderately important background variable Categorical: 0, 1, 2-3, 4-5, >5
Multiple birth	FetusNumber	Moderately important background variable. Categorical: Multiple birth=1; Singleton=0.
Maternal infection	MaternalPyrexiaInLabour38c, IntrapartumAntibioticsGiven, Chorioamnionitis.	Moderately important background variable. Binary: Infection=1, No infection=0 (Coded as 1 if any of MaternalPyrexiaInLabour38c, IntrapartumAntibioticsGiven, Chorioamnionitis are coded as 'Yes' in NNRD)
Antenatal steroids	SteroidsAntenatalGiven	Moderately important background variable. Binary: Any antenatal steroids given during pregnancy (partial or complete course) =1; No antenatal steroids given=0.
Illness severity	InotropesGiven, DrugsDay, RespiratorySupport, NitricOxide	Moderately important background variable. Categorical Illness severity score derived from the sum of: 1. Did the infant receive inotropes on either day 1 or 2 (coded as 1 or 0) (NNRD field: InotropesGiven OR any of the following drugs listed in the DrugsDay field: Adrenaline, Dopamine, Dobutamine, Milrinone, Noradrenaline or Vasopressin)

		Did the infant receive any invasive respiratory support on either day 1 or 2 (coded as 1 or 0) (NNRD fields: RespiratorySupport) 3. Did the infant receive any nitric oxide on either day 1 or 2 (coded as 1 or 0) (NNRD field: NitricOxide)
Intrauterine growth restriction (IUGR)	BW_UKWHO	Moderately important background variable. Binary: IUGR=1, No IUGR=0 (Coded as 1 if birthweight-for-age z-score <-2SD. Derived from birthweight z-score calculated against reference cohort [18])
Surfactant	SurfactantGivenResuscitation, DrugsDaily	Moderately important background variable. Binary: Received pulmonary surfactant = 1, Did not receive surfactant = 0 (Coded as 1 if surfactant is given either in the delivery room or on the neonatal unit on the day of birth).
Enteral feeds	DayEnteralFeeds	Moderately important background variable. Binary: Nil by mouth=1, Fed enterally =0 (Coded as 1 if infant was fed enterally in the first four days after birth).
Gut ischaemia	n/a	Not recorded in NNRD
Pre-eclampsia	ProblemsDuringPregnancy	Moderately important background variable. Binary: Pre-eclampsia present=1, Pre-eclampsia absent =0.
Placental abruption	ProblemsDuringPregnancy	Moderately important background variable. Binary: Abruption occurred=1, No abruption=0.
Mode of delivery	ModeOfDelivery	Moderately important background variable. Binary: Vaginal=0; Caesarean section=1; Missing.
Perinatal hypoxia	MethodsOfResuscitation	Moderately important background variable. Binary: Perinatal hypoxia=1; No perinatal hypoxia=0 (Coded as 1 if infant required significant resuscitation at birth, defined as one or more of cardiac compressions, adrenaline or other drugs.

Figure 1: Directed acyclic graph summarising critical relationships involved in the association between probiotics and severe NEC.



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SUPPLEMENTARY MATERIAL 1: MAJOR CONGENITAL ABNORMALITIES

Major congenital gastrointestinal malformations

Correction of congenital atresia of oesophagus, oesophageal atresia, oesophageal atresia with distal tracheo-oesophageal fistula, oesophageal atresia with tracheoesophageal fistula, oesophageal atresia without distal fistula, oesophageal atresia without tracheoesophageal fistula, thoracotomy and repair of oesophageal atresia and tracheo-oesophageal fistula with primary anastomosis, atresia and stenosis of small intestine, atresia and stenosis of duodenum, duodenal atresia / stenosis / web (specify), duodenal atresia / stenosis / web, duodenal atresia / stenosis, duodenal atresia, atresia and stenosis of ileum, ileal atresia / stenosis (specify), ileal atresia / stenosis, jejunal atresia / stenosis (specify), jejunal atresia / stenosis, atresia and stenosis of large intestine, congenital absence atresia / stenosis parts of large intestine, congenital absence atresia / stenosis parts of large intestine, congenital absence atresia / stenosis of rectum with fistula, congenital absence atresia / stenosis rectum without fistula, congenital absence atresia / stenosis anus with fistula, congenital absence atresia / stenosis anus without fistula, congenital absence atresia / stenosis of large intestine part unspecified, atresia of oesophagus without fistula, atresia of oesophagus with tracheo-oesophageal fistula (tof), recurrent tracheo-oesophageal fistula, tracheo-oesophageal fistula (h-type), congenital tracheo-oesophageal fistula without atresia (tof), congenital stenosis and stricture of oesophagus, congenital stenosis of the oesophagus, congenital oesophageal web, oesophageal web, large bowel or rectum - atresia, high anorectal anomaly with rectourethral fistula, high anorectal anomaly with rectovesical fistula, high anorectal anomaly with rectocutaneous fistula, high anorectal anomaly with rectocloacal fistula, high anorectal anomaly with fistula (specify), high anorectal anomaly without fistula, anorectal anomaly - high without fistula, low anorectal anomaly with anocutaneous fistula, low anorectal anomaly with anovestibular fistula, low anorectal anomaly with fistula (other specify), congenital absence atresia / stenosis anus without fistula, anus - imperforate, imperforate anus, low anorectal anomaly without fistula, low anorectal anoma, congenital anal stenosis, persistent cloaca, exomphalos (major), exomphalos (minor), exomphalos malrotation, exomphalos, omphalocele, closure of gastroschisis includes closure of exomphalos, primary repair exomphalos, repair exomphalos using prosthesis (specify type), gastroschisis, delayed closure gastroschisis, primary repair gastroschisis, repair gastroschisis using prosthesis (specify type), silo insertion for reduction of gastroschisis, delayed closure exomphalos, cutback of covered anus, repair of imperforate anus (with or without vaginal, cutback of low anorectal anomaly (nixon), oesophageal atresia - repair of anastomotic leak, primary repair of oesophageal atresia, closure of recurrent tracheo-oesophageal fistula, closure of tracheooesophageal fistula, closure of tracheo-oesophageal fistula, duodenal atresia/stenosis repair, duodenal atresia/stenosis repair (von)

Other severe congenital conditions, lethal or requiring early surgical intervention

Cardiac and circulatory system

Congenital malformations of cardiac chambers and connections, common arterial trunk (truncus malformation), truncus arteriosus, double outlet right ventricle (dorv), double outlet left ventricle (dolv), dextrotransposition of aorta, transposition great arteries (tga), transposition of the great vessels (tga), double inlet ventricle (dilv), discordant atrioventricular connection, isomerism of atrial appendages, atrial isomerism & asplenia, atrial isomerism with asplenia, atrial isomerism with polyspenia, atrial isomerism, other congenital malforms of cardiac chambers and connections, congenital malforms of cardiac chambers and connections unspec, complete atrioventricular septal

defect, atrio-ventricular septal defect (avsd), atrioventricular septal defect (avsd), tetralogy of fallot, atrium single, ventricle single, congenital malformations of pulmonary and tricuspid valves, pulmonary valve atresia, congenital pulmonary valve stenosis, pulmonary valve stenosis (ps), congenital pulmonary valve insufficiency, other congenital malformations of pulmonary valve, congenital tricuspid atresia / stenosis, ebstein's anomaly, hypoplastic right heart syndrome, other congenital malformations of tricuspid valve, congenital malformation of tricuspid valve (unknown or unspecified cause), congenital malformations of aortic and mitral valves, congenital stenosis of aortic valve (as), bicuspid aortic valve, mitral atresia, congenital insufficiency of aortic valve, congenital mitral stenosis (ms), hypoplastic left heart syndrome (hlh), other congenital malformations of aortic and mitral valves, congenital malformation of aortic and mitral valves unspec, coarctation of aorta, coarctation of the aorta, stenosis of aorta (as), other malformation of aorta, malformation of aorta, double aortic arch, hypoplasia of aortic arch, interrupted aortic arch, atresia of pulmonary artery, pulmonary stenosis (physiological branch stenosis), pulmonary stenosis - branch, other congenital malformations of great arteries, total anomalous pulmonary venous connection (tapvd), total anomylous pulmonary venous drainage (tapvd), blalock-taussig shunt

Respiratory system, including diaphragmatic hernia

Choanal atresia - bilateral, choanal atresia - unilateral (l), choanal atresia - unilateral (r), choanal atresia / stenosis (specify), choanal stenosis, congenital malformations of trachea and bronchus, congenital tracheomalacia, tracheomalacia, other congenital malformations of trachea, tracheal agenesis or atresia, bronchomalacia, congenital malformations of bronchus, congenital cystic lung (ccam), congenital cystic lung (congenital lobar emphysema), congenital cystic lung, sequestration of lung, congenital bronchiectasis, hypoplasia and dysplasia of lung, repair choanal atresia, congenital diaphragmatic hernia, congenital diaphragic hernia, morgagni diaphragmatic hernia, diaphragmatic hernia - left, diaphragmatic hernia - right, recurrent congenital diaphragmatic hernia, eventration of diaphragic hernia, eventration of the diaphragm, repair of congenital diaphragmatic hernia, prosthetic repair of congenital diaphragmatic hernia (specify), aplasia of the diaphragm, fetoscopic insertion of tracheal plug for congenital diaphragmatic hernia, other repair of diaphragmatic hernia (specify), other specified repair of diaphragmatic hernia, repair of diaphragmatic hernia using abdominal approach nec, primary repair of congenital diaphragmatic hernia, thoracoscopic repair of congenital diaphragmatic

Brain and nervous system

Does not include spina bifida occulta

Frontal encephalocele, nasofrontal encephalocele, occipital encephalocele, encephalocoele - occipital, encephalocele (unknown or unspecified cause), encephalocele, meningocele (specify site), myelomeningocele (specify site), meningocele & hydrocephalus (specify site), thoracic spina bifida with hydrocephalus, lumbar spina bifida with hydrocephalus, sacral spina bifida with hydrocephalus, (unknown or unspecified cause) spina bifida with hydrocephalus, cervical spina bifida without hydrocephalus, thoracic spina bifida without hydrocephalus, lumbar spina bifida without hydrocephalus, sacral spina bifida without hydrocephalus, spina bifida (unknown or unspecified cause), spina bifida, repair of spina bifida, repair of encephalocele, anencephaly and similar malformations, anencephaly, craniorachischisis, iniencephaly, holoprosencephaly, closure of spinal myelomeningocele, closure of spinal meningocele

Urinary system

Bilateral renal agenesis, renal agenesis, bilateral, potter's syndrome, autosomal recessive polycystic kidney - infantile, polycystic kidney, infantile type, autosomal dominant polycystic kidney in childhood, polycystic kidney, adult type, polycystic kidney, exstrophy of urinary bladder, bladder exstrophy, posterior urethral valves (puv), congenital posterior urethral valves (puv), congenital absence of bladder and urethra

Other miscellaneous lethal conditions

Thanatophoric short stature, edwards syndrome (trisomy 18), Edwards syndrome (unknown or unspecified cause), trisomy 18, Patau syndrome (trisomy 13), trisomy 13, sirenomelia, triploidy and polyploidy

SUPPLEMENTARY MATERIAL 2: DEFINITION OF NEC USING NNRD VARIABLES

A) Severe NEC

This method collects data from 3 tables:

- Episode
- Daily summary
- Abdoxray

Multiple fields are used to collect data about NEC diagnosis. We define Severe NEC as true if any of the following conditions (1-8) are met in any of the following tables:

Within the Episode table:

1. NEC is listed as 'Cause of Death'
2. 'Postmortem confirmation' is true.
3. Any of the following in 'Gastrointestinal Diagnoses', 'Principle Procedures during stay' or 'Principal Diagnosis at discharge':
    - LAPAROTOMY
    - LAPAROTOMY APPROACH NEC
    - COLECTOMY AND ILEOSTOMY NEC

    AND

    Any of the following in 'Gastrointestinal Diagnoses' or 'Principal Diagnosis at discharge':
    - NECROTISING ENTEROCOLITIS
    - NECROTIZING ENTEROCOLITIS
    - NECROTIZING ENTEROCOLITIS – CONFIRMED
    - NECROTISING ENTEROCOLITIS – CONFIRMED
    - NECROTISING ENTEROCOLITIS – PERFORATED
    - NECROTIZING ENTEROCOLITIS – PERFORATED
    - NECROTISING ENTEROCOLITIS - PROVEN (ON XRAY OR AT SURGERY)
    - NECROTIZING ENTEROCOLITIS - PROVEN (ON XRAY OR AT SURGERY

4. Any of the following in 'Gastrointestinal Diagnoses' or 'Principal Diagnosis at discharge':
    - NECROTISING ENTEROCOLITIS
    - NECROTIZING ENTEROCOLITIS
    - NECROTIZING ENTEROCOLITIS – CONFIRMED
    - NECROTISING ENTEROCOLITIS – CONFIRMED
    - NECROTISING ENTEROCOLITIS – PERFORATED
    - NECROTIZING ENTEROCOLITIS – PERFORATED
    - NECROTISING ENTEROCOLITIS - PROVEN (ON XRAY OR AT SURGERY)
    - NECROTIZING ENTEROCOLITIS - PROVEN (ON XRAY OR AT SURGERY

    AND

    'Discharge Destination' listed as Death.

5. Any of the following in 'Gastrointestinal Diagnoses' or 'Principal Diagnosis at discharge':
   NECROTISING ENTEROCOLITIS – PERFORATED
   NECROTIZING ENTEROCOLITIS – PERFORATED

Within the Daily summary table

6. Surgery is listed on any day in 'NEC Treatment'.

AND

Within the Episodes table

Any of the following in 'Gastrointestinal Diagnoses' or 'Principal Diagnosis at discharge':

- NECROTISING ENTEROCOLITIS
- NECROTIZING ENTEROCOLITIS
- NECROTIZING ENTEROCOLITIS – CONFIRMED
- NECROTISING ENTEROCOLITIS – CONFIRMED
- NECROTISING ENTEROCOLITIS – PERFORATED
- NECROTIZING ENTEROCOLITIS – PERFORATED
- NECROTISING ENTEROCOLITIS - PROVEN (ON XRAY OR AT SURGERY)
- NECROTIZING ENTEROCOLITIS - PROVEN (ON XRAY OR AT SURGERY
-

Within the Abdoxray table

7. 'Yes' is listed under 'Laparotomy Performed' and 'Yes' listed in 'Histology Confirmation NEC'
8. 'Yes' listed in 'Visual Inspection Confirmation NEC'

B) NNAP defined NEC

The NNAP definition of NEC, first ensures infants are born at less than 32 weeks gestation and survived to 48 hours using data from the Episodes table (Variables admittimeanon and dischtimeanon).

Once the cohort of infants is established, we categorised NEC using the 'NEC diagnosed at discharge' fields from the Episodes table.

/*Confirmed NEC*/

*NEC diagnosis confirmed by clinical signs;

if NECDiagnosis = **1** and NECDiagBasedOn =**10** and clinicalfeatures ^= **.** and radiographicfeatures ^ = **.** then NEC=**8**;

if NECDiagnosis = **1** and NECDiagBasedOn =**11** then NEC=**8**;* NEC confirmed by surgery;

if NECDiagnosis = **1** and NECDiagBasedOn =**12** then NEC=**8**; *NEC confirmed by post mortem;

if NECDiagBasedOn=**10** and clinicalfeatures ^= **.** and radiographicfeatures ^ = **.** then NEC=**8**; *clinical signs;

if NECDiagBasedOn in (**11**,**12**) then NEC=**8**; *postmortem or surgery;

/*No NEC*/

if NECDiagnosis =**0** then do;

if finaldischarge = **3** then NEC=**7**; *No NEC but died;

if finaldischarge ^=**3** then NEC=**6**; *No NEC but didnt die;

Where NECdiagnosis is ticked yes but missing clinical/radiographic features confirmation this was treated as No NEC.

Where NEC diagnosis is ticked yes but no further basis for that diagnosis, treated as no NEC

if NECDiagnosis = **1** and NECDiagBasedOn = **.** and finaldischarge= **3** then NEC=**4**; /*Where infant died*/

Infants with no NEC data (NNRD fields NECDiagnosis and NECDiagBasedOn) entered are treated as 'Missing'

Once an episodic view of the NEC variables has been captured, it is now necessary to look across all the episodes of a infant for a confirmed NEC diagnosis:

Maximise NEC status over all episodes for each infant. Coded as

Missing if NEC_Diagnosis = **0** then Final_NEC=**0**; OR if NEC_Diagnosis = **1** then Final_NEC=**1**;

NO NEC if NEC_Diagnosis in (**2**,**3**,**6**) then Final_NEC=**2**; OR if NEC_Diagnosis in (**4**,**5**,**7**) then Final_NEC=**3**; /*No NEC but died*/

NEC present if NEC_Diagnosis = **8** then Final_NEC=**4**

C) Pragmatic NEC

We define pragmatic NEC as present if either of the following conditions are met in any of the following tables:

Within the Daily summary table:

1. Surgery or conservative treatment is listed on any day in 'NEC Treatment'
   AND
   Being nil by mouth (confirmed by 'DayEnteralFeeds', 'FormulaName', 'FeedingMethod', 'VolumeMilk') and any of the following in 'DrugsDay' for 5 **consecutive** days:
   - 'BENZYL PENICILLIN'
   - 'AUGMENTIN'
   - 'FLUCLOXICILLIN'
   - 'FLUCLOXACILLIN'
   - 'GENTAMICIN'
   - 'CO-AMOXICLAV'
   - 'COAMOXICLAV'
   - 'CIPROFLOXACIN'
   - 'NETILMICIN'

- 'AMIKACIN'
- 'TAZOCIN'
- 'METRONIDAZOLE'
- 'VANCOMYCIN'
- 'CEFOTAXIME'
- 'AMPICILLIN'
- 'CEFUROXIME'
- 'CEFTAZIDIME'
- 'CEFTRIAXONE'
- 'PIPERACILLIN'
- 'OFLACILLIN'
- 'AZLOCILLIN'
- 'LINEZOLID'
- 'CEFALEXIN'
- 'AMOXICILLIN'
- 'MEROPENEM'
- 'IMEPENEM'
- 'IMIPENEM'

Within the Daily summary table:

2. Any of the following in 'DiagnosesDay':
   - NECROTISING ENTEROCOLITIS*
   - NECROTIZING ENTEROCOLITIS*

   OR

   Any of the following in 'Code' in the Diagnosis table:
   - **1010683**
   - **10708**
   - **15809**

   AND

   Being nil by mouth (confirmed by 'DayEnteralFeeds', 'FormulaName', 'FeedingMethod', 'VolumeMilk') and any of the following in 'DrugsDay' for 5 **consecutive** days:
   - 'BENZYL PENICILLIN'
   - 'AUGMENTIN'
   - 'FLUCLOXICILLIN'
   - 'FLUCLOXACILLIN'
   - 'GENTAMICIN'
   - 'CO-AMOXICLAV'
   - 'COAMOXICLAV'
   - 'CIPROFLOXACIN'
   - 'NETILMICIN'
   - 'AMIKACIN'
   - 'TAZOCIN'

- 'METRONIDAZOLE'
- 'VANCOMYCIN'
- 'CEFOTAXIME'
- 'AMPICILLIN'
- 'CEFUROXIME'
- 'CEFTAZIDIME'
- 'CEFTRIAXONE'
- 'PIPERACILLIN'
- 'OFLACILLIN'
- 'AZLOCILLIN'
- 'LINEZOLID'
- 'CEFALEXIN'
- 'AMOXICILLIN'
- 'MEROPENEM'
- 'IMEPENEM'
- 'IMIPENEM'

SUPPLEMENTARY MATERIAL 3: DEFINITIONS OF OTHER SECONDARY OUTCOMES

These will be defined as:

- Survival without severe NEC where severe NEC is defined as per the study's primary outcome
- Survival without severe NEC or late onset sepsis where severe NEC is defined as per the study's primary outcome and late onset sepsis is defined as per the study's secondary outcome.
- Survival without any NEC where NEC is defined as per the study's secondary outcome, NNAP defined NEC.
- Severe brain injury (defined as either left or right grade 3 or 4 intra-ventricular haemorrhage or cystic periventricular leukomalacia)
- Treated retinopathy of prematurity (defined as cryotherapy, laser therapy or injection of anti-vascular endothelial growth factor therapy for ROP in either or both eyes).
- Bronchopulmonary dysplasia (defined as any respiratory or ventilatory support or supplemental oxygen at 36 weeks postmenstrual age)
- Severe bronchopulmonary dysplasia (defined as ventilation via endotracheal tube or tracheostomy, and excluding non-invasive support or CPAP, at 36 weeks postmenstrual age)
- Time to full feeds: defined as the day of life when the infant first has three consecutive days without any parenteral nutrition or fluid (i.e. no parenteral nutrition or intravenous dextrose);

SUPPLEMENTARY MATERIAL 4: CODE LIST FOR THE DEFINITION OF LATE ONSET SEPSIS IN THE NNRD FIELD "PrincipalDiagnosesAtDischarge"

sepsis - confirmed bacterial (gram positive)
sepsis / septicaemia - confirmed with +ve microbiology
e.coli sepsis / septicaemia
candida sepsis / septicaemia
group b streptococcal sepsis / septicaemia (gbs)
staphylococcal sepsis / septicaemia
staph. aureus sepsis / septicaemia
sepsis / septicaemia - specified - klebsiella sp.
sepsis / septicaemia - specified - enterobacter sp.
sepsis / septicaemia - specified - pseudomonas sp.
extended beta lactamase coliform infection/sepsis
listeria sepsis / septicaemia / disseminated
sepsis - confirmed bacterial (streptococci b positive)
sepsis - confirmed bacterial (streptococci positive)
streptococcal sepsis / septicaemia
salmonella sepsis
sepsis due to streptococcus
umbilical sepsis / septicaemia- group b streptococcus

SUPPLEMENTARY MATERIAL 5: BUILDING THE PROPENSITY SCORE MODEL

In this study we will match pairs of infants on their propensity score (a linear function of the covariates that were included in the propensity score model). Following the DAG, we would also have included neonatal unit as a highly important variable. However, probiotics are a prophylactic and therefore in units that use probiotics, all infants exposed to the same intervention. Consequently it will be very difficult to find matches who were not exposed to probiotics within the same unit. Importantly, infants who are not given probiotics in a probiotic centre are likely to have other very different characteristics e.g. be sicker, less stable or on a palliative care pathway. To control for some important unit level differences we choose instead to include two variables, Level of Care (intensive care, high dependency care, special care or normal care) and hospital network, as highly important variables.

Logistic regression models will be fitted with all the critically important and highly important variables plus each of the moderately important variables added individually. The model with the largest value of the chi-squared statistic will be adopted if the test statistic exceeded 1.0. This cycle will be repeated, adding each remaining moderately important background variable individually, until none of the chi-squared test statistics exceed 1 or until all variables have been included in the model. We will assess the model containing the main effects for evidence of collinearity. Any variables where the variance inflation factors exceed five will be excluded.

Interactions between background variables will also be included in the propensity score model. To identify the interactions for inclusion, we will sort the main effects included in the model by the absolute value of their t-ratios. Starting with the variable with the highest t-ratio we will examine all potential interactions with that variable. Continuous variables may interact with themselves, but binary and categorical variables may not. Potential interactions will be added individually to the model and the two interactions with the largest value of the chi-squared statistic, are selected if the test statistic exceeds 2.71 (implying significance at the 5% level for a two tailed test with 1 degree of freedom). This process will be repeated for each of the main effects already selected for inclusion in the propensity score model.