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Early antibiotic use and incidence of necrotising enterocolitis in very preterm infants: A UK based observational study using routinely recorded data

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ABSTRACT

Introduction

Necrotising enterocolitis (NEC) remains a major contributor to preterm mortality and morbidity. Prolonged duration of antibiotic therapy after delivery is associated with later NEC development but recent evidence suggests that absence of antibiotic treatment after delivery may also increase NEC risk. We will explore this controversy using a large pre-existing dataset of preterm infants in the United Kingdom (UK).

Methods and analysis

This is a retrospective cohort study using routine data from the UK National Neonatal Research Database (NNRD) for infants born 01/01/2012 to 31/12/2020. Eligible infants will be <32 weeks gestation, alive on day three. The primary outcome is development of severe NEC, compared in infants receiving early antibiotics (days 1-2 after birth) and those not. Subgroup analysis on duration of early antibiotic exposure will also occur. Secondary outcomes are: late onset sepsis (LOS), total antibiotic use, pre-discharge mortality, retinopathy of prematurity (ROP), intraventricular haemorrhage (IVH), bronchopulmonary dysplasia (BPD), focal intestinal perforation and any abdominal surgery. To address competing risks, incidence of death before day 7, 14 and 28 will be analysed. We will perform both logistic regression and propensity score matched analyses. Statistical analyses will be guided by understanding of NEC risk factors, exposures and outcome presented in a causal diagram. These covariates include but are not limited to gestational age, birth weight, small for gestational age (SGA), sex, ethnicity, delivery mode, delivery without labour, Apgar score, early feeding and probiotic use. Sensitivity analyses of alternate NEC definitions, specific antibiotics and time of initiation will occur.

Ethics and dissemination

We will use de-identified data from NNRD, which holds permissions for the original data, from which parents can opt out and seek study- specific Research Ethics approval. The results will help to determine optimal use of early antibiotics for very preterm infants.

ARTICLE SUMMARY

Strengths and limitations of the study

Strengths:

- Use of the NNRD gives access to a very large dataset of preterm infants
- The primary outcome (NEC) and the many contributory covariates are routinely recorded in this dataset
- Analysis by both regression and using propensity matching optimises learning from this large dataset

Limitations:

- Data entry may not always be as accurate as that collected specifically within a trial
- The diagnosis of NEC has no gold standard to allow standardisation across units

INTRODUCTION

Around 3% of all babies are born very preterm (VPT, <32 weeks' gestation) and they require prolonged hospital stay, commonly including intensive care. Survival in these VPT infants (VPTI) has increased dramatically in recent years, but death is still common (~10% overall) as are life-long physical and cognitive impairment.[1] In the UK around 10,000 VPTI are born every year, representing an annual cost to the NHS of ~£3 billion.[2] The commonest cause of death or serious illness in preterm infants after the first few days are gut or infectious complications such as necrotising enterocolitis (NEC) or late onset sepsis (LOS).[3] Although knowledge around NEC, and preventive practices such as use of mothers own milk (MOM), donor human milk (DHM) and probiotics are increasing, there has been little reduction in NEC incidence over recent years[4,5], and mechanisms underlying the development of NEC are poorly understood. Antibiotic use as part of neonatal intensive care is common, particularly immediately after birth when infection is implicated in preterm delivery – studies show more than half of infants weighing <1000g routinely received more than 5 days antibiotics at birth[6]. Antibiotic use in VPTI has been implicated in NEC development in several ways. Studies show an increase in NEC incidence with increased duration of empirical early antibiotics[7,8] and alteration of the gut microbiotia (dysbiosis) has been mechanistically linked to NEC development [9]. However recent observational data from 13 NICUs from 5 continents (n=2831) identified that NEC incidence was higher in infants who did not receive empirical antibiotics early after birth, despite higher gestational age, compared to those receiving them (OR: 1.8 (95% CI 1.1-2.9), with even higher odds ratio when adjusted for relevant confounders (OR: 4.0 (95% CI 2.1-7.3)) [10]. In contrast, results from a very recent study in preterm infants with low risk of infection shows opposite trends of lower odds in those not treated, but is underpowered for NEC as outcome (n=641, OR:0.7 (95%CI 0.3-1.5))[11]. There is increasing focus on antibiotic stewardship, and it can be

expected that the proportion of infants that are not given antibiotics after preterm birth will increase in the coming years. Therefore, it is important to know if lower early usage of antibiotics will increase the incidence of NEC. While there have been calls for a trial of routine early antibiotic treatment[12] in very preterm babies, and a single trial has so far attempted to do this, there are important logistical difficulties[13] with such an approach. The National Neonatal Research Database (NNRD) provides a large, population level dataset that can be used to further test the hypothesis that early empiric antibiotic treatment reduces the incidence of NEC in preterm infants, and allows adjustment for confounding through the large number of patient level covariates recorded in the NNRD.

METHODS AND ANALYSIS

Design

Retrospective cohort study using routinely recorded clinical data held in the NNRD.

Data source

NNRD holds data from all infants admitted to National Health Service (NHS) neonatal units in England and Wales from around 90,000 infants annually. Neonatal units in England and Wales have contributed data since 2012. Data are entered by contributing units to a point-of-care electronic dataset and a defined dataset is extracted by NNRD. Data is extracted quarterly and sent to the Neonatal Data Analysis Unit (NDAU), based at Imperial College, London [14]. The data includes variables pertinent to the present analysis, including demographics, exposure and outcome variables.

Eligibility criteria

Eligible infants must have been born at <32 weeks gestation, be cared for in a unit contributing data to NNRD, and be alive at day 3. Infants will be excluded if they have a known severe congenital or gastro-intestinal anomaly (excluding the presence of a patent

ductus arteriosus (Supplementary Tables 1 and 2) or have had abdominal surgery before day 3.

Time period

Infants born between 01/01/2012 and 31/12/2020 will be included.

Setting

UK neonatal units in England and Wales contributing to NNRD.

Definitions

Exposure (primary)

Receipt of any intravenous antibiotic drug (Appendix 1) for any of the first 2 days after birth.

Comparator: Did not receive any antibiotics for any of the first 2 days after birth.

Primary outcomes

Severe NEC resulting in death or surgery as defined by Battersby[4].

Secondary outcomes

Secondary outcomes for analysis are the effects of early antibiotic exposure on

1. Late onset sepsis (blood stream or cerebrospinal fluid (CSF) confirmed pure growth in culture (National Neonatal Audit Programme (NNAP) definition) after first 3 days and/or treatment with 5 days of antibiotics and a concurrent diagnosis of infection after the first 3 days)
2. Total antibiotic use (number of days with any treatment of antibiotics during admission)
3. Length of stay (postnatal age at discharge or death)
4. Time to reach full feeding (first day of 3 consecutive days where parenteral nutrition or intravenous fluid are not recorded

5. Growth (change in standard deviation score between birth and 36 weeks and discharge)

Further, we will analyse effects on some relevant adverse outcomes:

6. Total pre-discharge mortality

7. Death prior to day 7, day 14, day 28

8. Bronchopulmonary dysplasia (respiratory support given at 36 weeks)

9. Retinopathy of prematurity (ROP) (received treatment for ROP, according to NNRD definition)

10. Brain injury (Intraventricular haemorrhage grade 3 or above or cystic leucomalacia diagnoses recorded)

11. Need for surgical procedures (Appendix 1)

Comparison of different durations of early antibiotic exposure will be performed based on the following categories:

- Antibiotic duration no longer than 3 days
- Antibiotic duration 3-5 days
- Antibiotic duration longer than 5 days without positive culture (blood stream or CSF confirmed pure growth in culture (NNAP definition) in the first 3 days.

For the above analyses, infants with a positive blood or CSF culture in the first 3 days will be excluded.

A specific subgroup of interest are the infants that are considered to have low risk of early onset sepsis (EOS), specified as fulfilling all of the following prenatal characteristics: no premature rupture of membranes (PROM), no labour and no (suspected) chorioamnionitis.

Additional subgroup analyses will be performed for infants with gestation age <28 weeks and birth weight <1000g.

Sample size

Observed NEC incidence noted in a previous study on a total 2831 infants from five different continents, using criteria for NEC diagnosis in keeping with pragmatically defined NEC, was 9% when early antibiotic treatment was absent and 4% when antibiotic was provided in the first three days[10]. We hypothesise to find a similar antibiotic related proportional reduction in incidence of severe NEC in this study, based on data collected over nine years (2012-2020) from around 45,000 infants. In an earlier report based on a NNRD subgroup, the incidence of severe NEC was 3.2% for infants born <32 weeks[4]. The cohort event estimate is 1440 cases.

Data required

Appendix 1 carries the full list of variables considered relevant for extraction from NNRD including definitions of constructed items/variables.

Potential confounders

Several covariates are relevant to include in the analysis as potential confounders. We will take a hypothesis driven approach to the selection of covariates. A causal diagram (Directed Acyclic Graphs, DAG, Figure 1) is drawn and analysed with relevant variables and potential confounders related to antibiotic exposure and NEC outcome. Nodes and edges are determined based on literature and subject matter knowledge. The selected covariates are considered to reflect conditions prior to the defined exposure (i.e. within day 1-2 after birth). For several variables, only proxies will be available (Table 1).

Table 1 Overview of variables

Variable	Class/type	Expected availability in NNRD	Importance for effect estimation
AB	Exposure	Available (definable)	
NEC	Outcome	Available (definable)	
Site	Confounder	Available	Minimal sufficient adjustment set to model the direct and total effect
BW	Confounder	Available	
Delivery mode[1]	Confounder	Available (categories)	

Clinical signs[2]	Confounder	Available (proxies)	of AB on NEC according to proposed DAG
Maternal infection	Confounder	Available (clinical)	
GA	Confounder	Available	
IUGR	Confounder	Available (definable)	
Fetal flow	Ancestor of Exp & Out (indirect)	Unobserved	Blocked by IUGR and delivery mode
Ischemia	Ancestor of Exp & Out (indirect)	Unobserved	Blocked by Clinical Signs
Preeclampsia	Ancestor of Exp & Out (indirect)	Available	Blocked by delivery mode
Antenatal steroids	Ancestor of Outcome	Available	Blocked by clinical signs
Sepsis	Ancestor of Exp & Out (indirect)	Available (definable/proxy)	Blocked by clinical signs
PDA	Ancestor of Exp & Out (indirect)	Available (definable)	Blocked by clinical signs
Umbilical catheters	Ancestor of Exp & Out (indirect)	Available	Blocked by clinical signs
Anaemia/transfusion	Ancestor of Exp & Out (indirect)	Available (proxy, i.e. transfusions)	Blocked by clinical signs
Sex	Ancestor of Outcome	Available	Precision variable
Ethnicity	Ancestor of Outcome	Available	Precision variable
Multiparity	Ancestor of Outcome	Available	Precision variable
Smoking	Ancestor of Outcome	Available	Precision variable
GDM	Ancestor of Outcome	Available	Precision variable
Socioeconomic status	Ancestor of Outcome	Available (proxy i.e. deprivation score)	Precision variable
Maternal antibiotics	Ancestor of Outcome	Available (intrapartum)	Precision variable
Dyscolonisation	Ancestor of Outcome	Unobserved	Precision variable
Assisted ventilation	Ancestor of Outcome	Available	Precision variable
Surfactant therapy	Ancestor of Outcome	Available	Precision variable
Formula feeding	Ancestor of Outcome	Available	Precision variable
Feeding initiation	Ancestor of Outcome	Available	Precision variable
Probiotic initiation	Ancestor of Outcome	Available	Precision variable

[1]Specification of different clinical conditions with important impact on decision to treat with AB, categorized as: Vaginal AND Spontaneous, Vaginal AND Induced, Emergency caesarean AND labour, Emergency caesarean AND no labour, Elective caesarean AND labour, Elective caesarean AND no labour.
[2]Respiratory/circulatory/unspecific signs/symptoms/parameters used clinical assessment and decision making related to decision to treat with antibiotics

STATISTICAL ANALYSES

Primary analyses

Previous work using logistic regression included the following covariates in the model for the hypothesis: NICU (random effect) + GA + birthweight+ sex+ delivery mode + APGAR scores + antenatal steroids + feeding type. We aim to test the hypothesis with data from NNRD using the same regression model as used in the previous work (variables 1-8 in Table 2) and also an expanded regression model with inclusion of all potentially relevant variables (Table 2). Results will be presented as adjusted odds ratios with 97.5% confidence intervals and Bonferroni-adjusted p-values (unadjusted p-values multiplied by 2). To better quantify the causal effect of antibiotics, standardised risk differences with 97.5% bootstrap confidence intervals will also be presented.

Priority of covariates

Covariates to include in the model are listed and prioritised in Table 2. Confounders are ranked higher based on importance, i.e. variables which arguably have effect on both outcome (NEC) as well as exposure (decision to start antibiotic treatment, which relates to infection risk/concern). Assessment of covariate importance is based on subject matter knowledge and scientific literature (references in Table 2). For several variables, it is unclear whether there is a relevant effect on NEC and a conservative approach is employed to include potential confounders in the model[15]. Similar considerations apply for assessment of variables with relevant effect on decision to treat with early antibiotics. These variables will be included in the regression for propensity score calculation and subsequent matching. For highly similar variables, the lower priority or quality variables may be omitted if necessary (e.g multi-collinearity issues). Variables with very low quality (e.g. too many missing values)

will be omitted. For categorical variables, groups with very few observations will be removed (e.g. separation issues). Estimated effects of each variable included in the model included will be reported. Based on the recommendation to have at least ten events per variable[16], with the event estimate approximately 1500 cases, this will provide 150 degrees of freedom in the model. Based on the proposed covariates listed in Table 2, the required degrees of freedom for analysis is 108. If the actual number of cases in the obtained dataset is much lower than expected, thus providing insufficient degrees of freedom, covariates may be excluded in reverse order of priority. See detailed specifications of listed covariates/items in Appendix 1.

Table 2. Priority of covariates to include in model based on DAG and availability from NNRD.

		Influence on NEC[17–19]	**Influence on AB-start (decision to treat based on sepsis risk)**[20,21]	**Potential repetition/ redundancy**	**Relation to node in DAG**	**Structure (continuous or number of categories)**
1	**NICU/site**	YES	YES		Site	Random
2	**GA**	YES	YES		GA	Continuous
3	**BW**	YES	YES		BW	Continuous
4	**SEX**	YES	No?		Sex	Dichotomous
5	**APGAR5**	Yes	Yes		Clinical signs	11 categories (0-10)
6	**Delivery mode + expanded (6 categories)**	Yes	YES		Delivery mode and type	6 (see table 1)
7	**Maternal antenatal steroids**	Yes	Yes? (indicator of fetal status/delivery conditions)		Antenatal steroids	None / Incomplete / complete
8	**Feeding first day**	Yes	No		Feeding	1: Enteral feeding on day 1-2, human milk only 2: Enteral feeding on day 1-2, formula only 3: Enteral feeding on day 1-2, mix 3: No enteral feeding on day 1-2
9	IUGR	Yes	Yes		IUGR	Dichotomous (less than -2SDS)
10	APGAR1	Yes	Yes?			11 categories (0-10)
11	APGAR10	Yes	Yes		Clinical signs	11 categories (0-10)
12	EOS	Yes?	No		Sepsis	Dichotomous
13	Birth year (epoch)	Yes	Yes		(similar to site/standards)	4-5
14	Transfer on first day	Yes	Yes		Site/outborn	Dichotomous
15	Level of initial unit	Yes	Yes		Site	Dichotomous
16	Maternal Preeclampsia requiring preterm birth	Yes?	Yes		Preeclampsia	Dichotomous
17	Prolonged ROM	Yes?	YES		Maternal infection	Dichotomous
18	Maternal suspected chorioamnionitis	Yes?	YES	Defined by antibiotics and pyrexia	Maternal infection	Dichotomous
19	Intrapartum antibiotics	Yes?	YES (in relation to chorioamnionitis)		Maternal antibiotics	Dichotomous
20	Maternal pyrexia	Yes?	YES (untreated chorioamnionitis)		Maternal infection	Dichotomous
21	Maternal GBS	Yes?	YES		Maternal infection	Dichotomous
22	Umbilical cord pH	Yes	Yes		Clinical signs	Dichotomous: <7.00 yes or no
23	Umbilical cord lactate	Yes	Yes	Resembles pH	Clinical signs	Cont/ Di/tri?
24	Base excess 12h worst	Yes	Yes		Clinical signs	Dichotomous: < -5 yes/no
25	Umbilical cord base excess	Yes	Yes	Resembles BE 12h worst	Clinical signs	Dichotomous: < -5 yes/no

26	Blood transfusion day 1-2	Yes	Yes?		Anemia	Dichotomous
27	Chest compressions	Yes	Yes?		Clinical signs	Dichotomous
28	Resuscitation drugs at delivery	Yes	Yes?		Clinical signs	Dichotomous
29	Ventilation at delivery	Yes?	Yes? (clinical status at birth)		Assisted ventilation	Dichotomous
30	Spontaneous respiration time	Yes?	Yes?		Clinical signs	3 categories: <1 min, 1-5 min, >5 min
31	Admission temp	Yes	Yes?		Clinical signs	3 categories: <36.5, 36.5-37.5, >37.5
32	Admission oxygen SAT	Yes	Yes		Clinical signs	3 categories: >94, 90-94, <90
33	Inotropes on first day	Yes	Yes?		Clinical signs	Dichotomous
34	Admission mean BP	Yes?	Yes/no?	Resembles inotropes	Clinical signs	Dichotomous: below GA yes/no
35	Ethnicity	Yes	Yes? (risk of inf)		Ethnicity	4 categories as suggested in appendix
36	Maternal deprivation score	Yes?	Yes? (risk of inf)		SES	Deprivation centiles?
37	Intubation first day	?	Yes?		Assisted ventilation	Dichotomous
38	Intubation at delivery	?	Yes?	Resembles intubation d1	Assisted ventilation	Dichotomous
39	Surfactant first day	Yes?	Yes?		Surfactant therapy	Dichotomous
40	Surfactant at delivery	?	Yes?	Resembles intubation d1	Surfactant therapy	Dichotomous
41	Time of cord clamp	Yes/No??	Yes? (clinical status at birth)		Clinical signs	Dichotomous: >60 seconds yes/no
42	Probiotics	Yes	No		Probiotic initiation	Dichotomous
43	PDA identified day 1-2	Yes	No		PDA	Dichotomous
44	PDA treatment day 1-2	Yes	No		PDA	Dichotomous
45	Multiplicity	?	No?		Multiplicity	Dichotomous
46	Smoking	Yes?	No?		Smoking	Dichotomous
47	Parity	?	No?		Parity	Dichotomous
48	Umbilical catheters	Yes?	No		Umbilical catheters	Dichotomous
49	Parenteral nutrition d1-2	?	?			Dichotomous
50	Admission heart rate	?	?		Clinical signs	3 categories: >200, 100-200, <100
51	Maternal antenatal magnesium sulphate	No?	Yes/no ?	Resembles preeclampsia	Preeclampsia	Dichotomous
52	Maternal gestational hypertension	No?	No			Dichotomous
53	Maternal diabetes	No?	No		GDM	Dichotomous

Sensitivity analyses

The following sensitivity analyses will be performed:

Early antibiotic exposure only with ampicillin or penicillin plus gentamicin, early antibiotic exposure defined by other timings after birth (later initiation and lasting until 4-6 days after birth) and alternative methods for diagnosing NEC (as standards for NEC diagnosis are unclear). For the latter analyses, we will define and re-analyse NEC diagnosis as 'pragmatic NEC' (5 days of nil by mouth and antibiotics and a diagnostic code of NEC) and NEC including focal intestinal perforation diagnosis (FIP). This condition is sometimes difficult to separate from NEC. We will also record infants with laparotomy- confirmed FIP (intestinal

perforation, classified as non-NEC) in addition to the primary NEC (Battersby et al). definition. The statistical analyses will be repeated using propensity score matching (with propensity scores based on exposure regression), as an alternative approach to logistic regression.

Secondary analyses

We intend to use the same logistic regression models for secondary outcomes, as those specified for the primary outcome. The most important confounders (or proxies) for the secondary outcomes are included in this model. Detailed model specification for each specific secondary outcome as done for the primary outcome is beyond the aim and scope of this study (focusing on NEC). With propensity score matching, direct comparison between antibiotic exposure versus controls can in principle be performed for any outcome, assuming correct model specification for the propensity score.

Exploratory analyses

Additional non-defined exploratory analyses based on findings from the dataset may be performed.

Missing data

We assume that missing data occur randomly between groups and will be imputed ten-fold using multiple imputation by chained equations. Results will be pooled according to Rubin's rule.

Multiple testing

Adjusted P values will be reported with Bonferroni correction of the two primary analyses (along with corresponding 97.5% confidence intervals) and Benjamini-Hochberg adjusted P values from the secondary analyses. Post hoc exploratory analyses will be reported without adjustment of P values and should be interpreted with corresponding caution.

TRIAL REGISTRATION, ETHICS AND HRA

The study will be registered with International Standard Randomised Controlled Trials Number (ISRCTN) before opening and is sponsored by Newcastle Hospitals NHS Foundation Trust and the protocol with statistical analysis plan will be uploaded to the Open Science Framework website osf.io prior to data analysis initiation. We will apply for HRA/REC approvals. The study is observational and uses de-identified data that is already collected.

PATIENT PUBLIC INVOLVEMENT AND IMPORTANCE TO THE NHS

We have worked closely with parents on all our studies. The NEC UK parent group and other parent groups and representatives continue to assert that better understanding of NEC is a key priority. The NHS, parents and babies experience significant burden from NEC in terms of adverse outcome, prolonged hospitalisation, developmental impact and NHS costs. There is significant concern related to use of antibiotics in the neonatal population and it is important that studies help optimal use of early antibiotics.

DISCUSSION

This study aims to add relevant scientific information to an important clinical decision made for every preterm infant admitted to a neonatal unit: the use and duration of antibiotics in the absence of clear signs of bacteraemia or early onset sepsis. Cases of culture-proven early onset sepsis (EOS) are relatively few, with rates being one to seven per 1000 live births in high income countries[22]. There are potentially large numbers of infants where a clinical choice is available to withhold early antibiotic treatment. Data are currently conflicting as to the overall impact on NEC of receiving (or withholding) antibiotics in the first days of life. Early bacterial nature and load in the preterm gut has been linked to NEC development[23, 24]. Use of intravenous antibiotics shortly after birth may slow colonisation, allowing the gut immune system a short period of adaption that reduces the risk of TLR4 mediated NEC[25].

The integrity of the mucosal barrier has been shown to improve significantly in the first days after preterm birth in humans[26]. Thus, potentially only short duration of very early antibiotic treatment may be relevant for such effect, in contrast to prolonged treatment which have been shown to cause persistent gut dysbiosis[27] that may instead increase NEC risk[7,8]. Data from a piglet model of NEC suggests that antibiotic use is mechanistically linked to preterm NEC development[28] and preterm immune development[29]. However no difference was seen in total bacterial load of stool in preterm infants who did and did not go on to develop NEC[30]. Given the conflicting data Clinicians need better information to help guide early antibiotic treatment in relation to NEC, especially important as NEC rates in premature infants may actually be increasing[31]. The proposed study using NNRD benefits from access to large numbers of infants with recorded relevant risk factors and outcomes. Large datasets offer the advantage of including many NEC cases, and we anticipate around 1500 informative cases of NEC. These data are increasingly well validated by individual units at the point of data entry, but are potentially less well validated than infants with trial data collected within specific trials.

We have in this study given careful thought to handling confounding factors. Analysis of the current understanding of NEC and the use of directed acyclic graph to guide analysis have been undertaken to attempt to control for what are highly complex clinical factors[17-19]. As demonstrated in the DAG many factors, including those on a causal pathway to NEC, impact the decision to administer early antibiotics[20-21]. The aim to analyse this data using both propensity scoring and logistic regression is a major strength for this study and for future analyses using large databases to address complex questions. Propensity scoring has recently been used to address feeding during hypothermia[32] and the impact of early parenteral nutrition on preterm outcomes[33] using the NNRD, but without alternate statistical approach. Whilst both propensity scoring and regression analysis have strengths and

weaknesses to the best of our knowledge direct comparison of these methodologies has not been undertaken within large neonatal datasets, and is important methodologically for future neonatal studies. The data generated by this study will thus inform important aspects of wider neonatal care and in relation to early neonatal use of antibiotics and later occurrence of NEC.

AUTHOR CONTRIBUTIONS

NE had the original idea for the study. RS undertook the DAG, RS,JF,JB, PTS planned statistical analysis. All authors have contributed to overall study design, protocol development and the writing and review of this paper.

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COMPETING INTERESTS

No author has relevant competing interests to declare.

DATA STATEMENT

Data may be available on request to the corresponding author.

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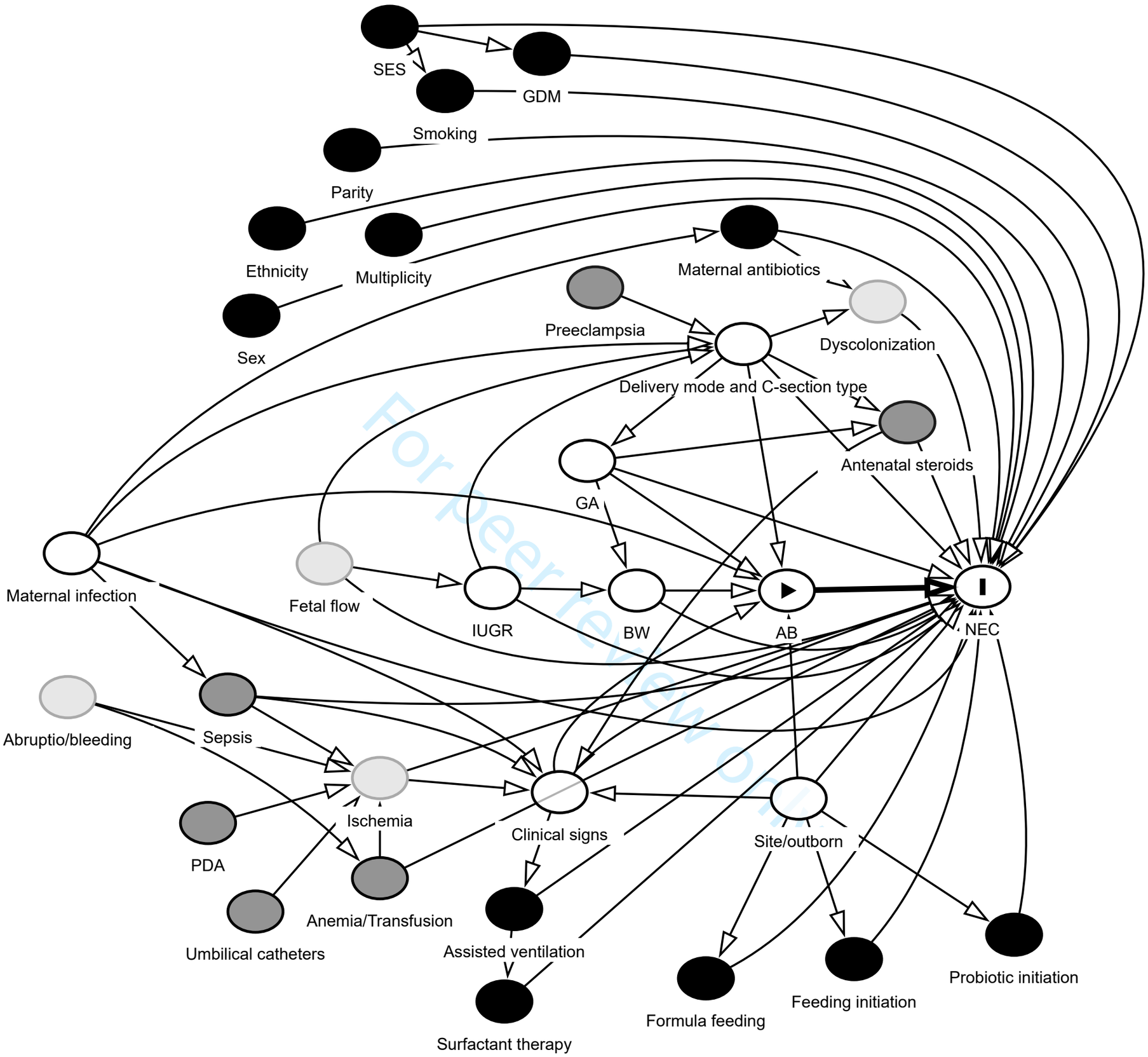
FIGURE LEGEND

Figure 1. Directed Acyclic Graph (DAG) diagram of causal assumptions related to the hypothesis based on subject-matter knowledge, used for confounder selection. Model code text for figure and interactive diagram analysis on dagitty.net is available in Appendix 2. Node with arrowhead: exposure; Node with I: outcome; Black nodes: ancestor of outcome; Dark grey nodes: ancestor of exposure and outcome; White nodes: adjusted variables (primary analysis); Thick arrow: causal path; Thin arrows: non-biasing paths. AB: early antibiotics; BW: birth weight; GA: gestational age; GDM: gestational diabetes mellitus; IUGR: intrauterine growth restriction; NEC: necrotising enterocolitis; PDA: patent ductus arteriosus; SES: socioeconomic status



Clevermed code	ICD-10 code	Diagnosis
10741	Q39.0	Oesophageal atresia without distal fistula
16195	Q39.0	Atresia of oesophagus without fistula
10740	Q39.1	Oesophageal atresia with distal tracheo-oesophageal fistula
16196	Q39.1	Atresia of oesophagus with tracheo-oesophageal fistula (TOF)
16197	Q39.2	Congenital tracheo-oesophageal fistula without atresia (TOF)
10273	Q39.3	Congenital stenosis of the oesophagus
16198	Q39.3	Congenital stenosis and stricture of oesophagus
16199	Q39.4	Oesophageal web
10358	Q41.0	Duodenal atresia / stenosis / web (specify)
16212	Q41.0	Congenital absence, atresia and stenosis of duodenum
16213	Q41.0	DA Duodenal atresia / stenosis
10605	Q41.1	Jejunal atresia / stenosis (specify)
16214	Q41.1	JA Jejunal atresia / stenosis
10541	Q41.2	Ileal atresia / stenosis (specify)
16215	Q41.2	Congenital absence, atresia and stenosis of ileum
16216	Q41.2	IA Ileal atresia / stenosis
16217	Q41.X	Congenital absence, atresia and stenosis of small intestine
16218	Q42.0	Congenital absence, atresia and stenosis of rectum with fistula
10496	Q42.00	High anorectal anomaly with rectourethral fistula
10497	Q42.01	High anorectal anomaly with rectovesical fistula
10498	Q42.02	High anorectal anomaly with rectovulval fistula
10495	Q42.03	High anorectal anomaly with rectocutaneous fistula
10494	Q42.04	High anorectal anomaly with rectocloacal fistula
10493	Q42.08	High anorectal anomaly with fistula (specify)
10499	Q42.1	High anorectal anomaly without fistula
16219	Q42.1	Congenital absence, atresia and stenosis of rectum without fistula
16220	Q42.2	Congenital absence, atresia and stenosis of anus with fistula
10636	Q42.20	Low anorectal anomaly with anocutaneous fistula
10637	Q42.21	Low anorectal anomaly with anovestibular fistula
10638	Q42.28	Low anorectal anomaly with fistula (other specify)
10639	Q42.3	Low anorectal anomaly without fistula
16221	Q42.3	Congenital absence, atresia and stenosis of anus without fistula
10240	Q42.31	Congenital anal stenosis
16222	Q42.8	Congenital absence, atresia and stenosis of anus of other parts of large intestine
16223	Q429	Congenital absence, atresia and stenosis of anus of large intestine, part unspecified
16224	Q42X	Congenital absence, atresia and stenosis of large intestine
16235	Q43.7	Persistent cloaca

Supplementary Table 1 Gastrointestinal anomalies

Clevermed code	ICD-10 code	Diagnosis
15890	Q00.0	Anencephaly
15891	Q00.1	Craniorachischisis
15892	Q00.2	Iniencephaly
15893	Q00.X	Anencephaly and similar malformations
15894	Q01.0	Frontal encephalocele
15895	Q01.1	Nasofrontal encephalocele
15896	Q01.2	Occipital encephalocele
15897	Q01.8	Encephalocele of other sites
15898	Q01.9	Encephalocele (unknown or unspecified cause)
15899	Q01.X	Encephalocele
15918	Q04.2	Holoprosencephaly
15926	Q05.0	Cervical spina bifida with hydrocephalus
15927	Q05.1	Thoracic spina bifida with hydrocephalus
15928	Q05.2	Lumbar spina bifida with hydrocephalus
15929	Q05.3	Sacral spina bifida with hydrocephalus
15930	Q05.4	(unknown or unspecified cause) spina bifida with hydrocephalus
15931	Q05.5	Cervical spina bifida without hydrocephalus
15932	Q05.6	Thoracic spina bifida without hydrocephalus
15933	Q05.7	Lumbar spina bifida without hydrocephalus
15934	Q05.8	Sacral spina bifida without hydrocephalus
15935	Q05.9	Spina bifida (unknown or unspecified cause)
10986	Q05.9a	Spina bifida
10704	Q05.9b	Myelomeningocele (specify site)
15936	Q05.X	Spina bifida
16024	Q20.0	Common arterial trunk (Truncus malformation)
10356	Q20.1	Double outlet right ventricle (DORV)
16025	Q20.1	Double outlet right ventricle (DORV)
16026	Q20.2	Double outlet left ventricle (DOLV)
11070	Q20.3	Transposition of the great vessels (TGA)
16027	Q20.3	Transposition great arteries (TGA)
16028	Q20.4	Double inlet ventricle (DILV)
16029	Q20.5	Discordant atrioventricular connection
16030	Q20.6	Is omerism of atrial appendages
16031	Q20.8	Other cong malforms of cardiac chambers and connections
16032	Q20.9	Cong malforms of cardiac chambers and connections unspec
16033	Q20.X	Congenital malformations of cardiac chambers and connections
16035	Q20.91	Atrium single
16036	Q20.92	Ventricle single
10097	Q21.2	Atrio-ventricular septal defect (AVSD)
16039	Q21.2	Atrioventricular septal defect (AVSD)
11043	Q21.3	Tetralogy of Fallot
16040	Q21.3	Tetralogy of Fallot

16045	Q22.0	Pulmonary valve atresia
16046	Q22.1	Congenital pulmonary valve stenosis
16047	Q22.2	Congenital pulmonary valve insufficiency
16048	Q22.3	Other congenital malformations of pulmonary valve
16049	Q22.4	Congenital tricuspid atresia / stenosis
16050	Q22.5	Ebstein's anomaly
16051	Q22.6	Hypoplastic right heart syndrome
16052	Q22.8	Other congenital malformations of tricuspid valve
16053	Q22.9	Congenital malformation of tricuspid valve (unknown or unspecified cause)
16054	Q22.X	Congenital malformations of pulmonary and tricuspid valves
16055	Q23.0	Congenital stenosis of aortic valve (AS)
16056	Q23.1	Congenital insufficiency of aortic valve
16057	Q23.2	Congenital mitral stenosis (MS)
16058	Q23.3	Mitral atresia
16059	Q23.4	Hypoplastic left heart syndrome (HLH)
16060	Q23.8	Other congenital malformations of aortic and mitral valves
16061	Q23.9	Congenital malformation of aortic and mitral valves unspec
16062	Q23.X	Congenital malformations of aortic and mitral valves
16079	Q25.1	Coarctation of aorta
10227	Q25.19	Coarctation of the aorta
16080	Q25.2	Hypoplasia of aortic arch
16081	Q25.3	Stenosis of aorta (AS)
16082	Q25.4	Malformation of aorta
16083	Q25.5	Atresia of pulmonary artery
16084	Q25.6	Stenosis of pulmonary artery (PS)
16086	Q25.8	Other congenital malformations of great arteries
16087	Q25.8	Transposition of the great vessels (TGA)
11057	Q26.2	Total anomylous pulmonary venous drainage (TAPVD)
16092	Q26.2	Total anomalous pulmonary venous connection (TAPVD)
16154	Q33.6	Hypoplasia and dysplasia of lung
16241	Q44.2	Atresia of bile ducts
10123	Q60.1	Bilateral renal agenesis
16318	Q60.1B	Renal agenesis, bilateral
16324	Q60.6	Potter's syndrome
16327	Q61.1	Polycystic kidney, infantile type
10100	Q61.1a	Autosomal recessive polycystic kidney - infantile
10367	Q64.1	Ectopia vesicae
16356	Q64.1	Exstrophy of urinary bladder
10854	Q64.2	Posterior urethral valves (PUV)
16357	Q64.2	Congenital posterior urethral valves (PUV)
16360	Q64.5	Congenital absence of bladder and urethra
10008	Q64.5a	Absence of bladder
10236	Q64.5b	Congenital absence of urethra
16475	Q77.1	Thanatophoric short stature

10246	Q79.0	Congenital diaphragmatic hernia
10490	Q79.0	Hernia into the cord
16495	Q79.0	Congenital diaphragmatic hernia
16496	Q79.1A	Aplasia of diaphragm
16497	Q79.1E	Eventration of diaphragm
16498	Q79.2	Exomphalos
10395	Q79.2	Exomphalos
16499	Q79.3	Gastroschisis
16589	Q90.0	Trisomy 21, meiotic nondisjunction
16590	Q90.1	Trisomy 21, mosaicism (mitotic nondisjunction)
16591	Q90.2	Trisomy 21, translocation
16592	Q90.9	Down's syndrome (unknown or unspecified cause)
16593	Q90.X	Down's syndrome
16594	Q91.0	Trisomy 18, meiotic nondisjunction
16595	Q91.1	Trisomy 18, mosaicism (mitotic nondisjunction)
16596	Q91.2	Trisomy 18, translocation
16597	Q91.3	Edwards' syndrome (unknown or unspecified cause)
16598	Q91.4	Trisomy 13, meiotic nondisjunction
16599	Q91.5	Trisomy 13, mosaicism (mitotic nondisjunction)
16600	Q91.6	Trisomy 13, translocation
16601	Q91.7	Patau's syndrome (unknown or unspecified cause)
16602	Q91.X	Edwards' syndrome and Patau's syndrome

Supplementary Table 2 Congenital anomalies

Early antibiotic use and incidence of necrotising enterocolitis in very preterm infants: a UK based observational study using routinely recorded data

Inclusion criteria: Birth weight < 1500g or GA < 32 wk

Born between: 1/1/2012 and 31/12/2020

Request data as one table with individual-level data with each row representing a unique baby. Each item/variable as separate column.

Exposure parameters:

Requested item	Item name in extract	How is it derived	Coding in extract	Notes
Antibiotics any	Antibiotics_Day1	Postnatal Day 1 with any DailyDrugs in 1010155 Benzyl Penicillin 1010158 Augmentin 1010179 Flucloxicillin 500012 Flucloxacillin 500016 Gentamicin 500072 Co-amoxiclav 500086 Co-amoxiclav 500084 Ciprofloxacin 500029 Netilmicin 500002 Amikacin 500211 Tazocin 500023 Metronidazole 500040 Vancomycin 500007 Cefotaxime 500004 Ampicillin 500009 Cefuroxime 500008 Ceftazidime 500175 Ceftriaxone 500032 Piperacillin 500206 Oflacillin 500005 Azlocillin 1010171 Linezolid 1010271 Cefalexin 1010139 Amoxicillin 500070 Amoxicillin 500128 Meropenem 500118 Imepenem 500145 Imipenem THEN (1)	0 = No 1 = Yes 9 = Unknown	Indicates whether baby has received antibiotics on day 1
	Antibiotics_Day6	As above for each postnatal day between Day2-Day6	0 = No 1 = Yes	

			9 = Unknown	
Antibiotics standard empiric		As above, but only ampicillin or penicillin + gentamicin		
	Day of first antibiotics	Postnatal day of first (of any) antibiotic treatment	Numeric (age in days since birth if received antibiotics) 999 = No antibiotics received throughout admission	
	Total antibiotics	Total number of days on (any) antibiotics throughout admission	Numeric 999 = No antibiotics received throughout admission	

Outcome parameters

Requested item	**Item name in extract**	**How is it derived**	**Coding in extract**	**Notes**
Length of stay		Defined as the total number of days a baby received neonatal care (any level of care) from Daily Care General Information - LOCATIONS OF HIGHEST LEVEL OF CARE	Numeric (age in days at discharge)	
Survival	PostnatalDayofDeath	Where the following is true: DayDateAnon where DischargeDestination = 3	Numeric (age in days since birth if baby died) 999 = Survived	
	Death before day of interest	According to above - Before day 3 (exclusion) - Before day 7 (for competing risk analysis) - Before day 14 (for competing risk analysis) - Before day 28	0 = Yes 1 = No 9 = Unknown	
	SurvivaltoDischarge	Where the following is true: DischargeDestination = 3 THEN (0)	0 = Died 1 = Survived to discharge 9 = Unknown	Survival to discharge from neonatal care for the final episode
	FinalDischDestination	N/A	Text	Discharge destination for the final episode
Cause of death	Causeofdeath	ICD-10		
Necrotising enterocolitis (possible more than one episode)	NEC_NNAP	Where the following is true: NECTreatment > 0 AND XRayAppearances in	0 = No NEC 1 = NEC present 9 = Unknown	NNAP definition

		Pneumatosis Pneumoperitoneum AND ClinicalFindings in Increased/bilious aspirate Abdominal distension Bloody stools THEN (1)		
	Severe_NEC_Battersby	Where the following is true: CauseOfDeath = 17 OR PortMortemConfirmation = 1 OR GastrointestinalDiagnoses OR PrincipleProceduresDuringStay OR PrincipalDiagnosisAtDischarge in Laparotomy Laparotomy approach NEC Colectomy and ileostomy NEC AND GastrointestinalDiagnosis or PrincipalDiagnosisAtDischarge in Necrotising enterocolitis Necrotising enterocolitis – confirmed Necrotising enterocolitis – perforated Necrotising enterocolitis – proven (on xray or surgery) OR GastrointestinalDiagnoses OR PrincipalDiagnosisAtDischarge in Necrotising enterocolitis Necrotising enterocolitis – confirmed Necrotising enterocolitis – perforated Necrotising enterocolitis – proven (on xray or surgery) AND DischargeDestination = 3 OR GastrointestinalDiagnoses or PrincipalDiagnosisAtDischarge = Necrotising enterocolitis – perforated OR NECTreatment >= 1 AND GastrointestinalDiagnoses OR PrincipalDiagnosisAtDischarge in Necrotising enterocolitis Necrotising enterocolitis – confirmed Nectotising enterocolitis – perforated Necrotising enterocolitis – proven (on xray or surgery)	0 = No severe NEC 1 = Severe NEC present 9 = Unknown	Battersby Severe NEC definition

		OR LaparotomyPerformed = Yes AND HistologyConfirmationNEC = Yes OR VisualInspectionConfirmationNEC = Yes THEN (1)		
	NEC_Any (pragmatic)	Where the following is true: (TreatmentNEC >= 1 OR Code in 1010683 Necrotising enterocolitis – suspected 10708 Necrotising enterocolitis – Perforated 15809 Necrotizing enterocolitis 15809 Necrotising enterocolitis - Confirmed) AND (5 or more days of nil by mouth where DayEnteralFeeds = 0 OR DayFormulaType = No entry OR VolumeMilk = 0/No entry AND 5 consecutive days of antibiotics over the same five days of receiving nil by mouth where DailyDrugs in 1010155 Benzyl Penicillin 1010158 Augmentin 1010179 Flucloxicillin 500012 Flucloxacillin 500016 Gentamicin 500072 Co-amoxiclav 500086 Co-amoxiclav 500084 Ciprofloxacin 500029 Netilmicin 500002 Amikacin 500211 Tazocin 500023 Metronidazole 500040 Vancomycin 500007 Cefotaxime 500004 Ampicillin 500009 Cefuroxime 500008 Ceftazidime 500175 Ceftriaxone 500032 Piperacillin	0 = No NEC present 1 = NEC present 9 = Unknown	Webbe NEC definition for any NEC treatment

		500206 Oflacillin 500005 Azlocillin 1010171 Linezolid 1010271 Cefalexin 1010139 Amoxicillin 500070 Amoxicillin 500128 Meropenem 500118 Imepenem 500145 Imipenem OR Died within 5 days of ' TreatmentNEC >= 1')		
	NEC onset day	Postnatal age for NEC onset TreatmentNEC >= 1 OR First day where diagnosis 1010683 Necrotising enterocolitis – suspected 10708 Necrotising enterocolitis – Perforated 15809 Necrotizing enterocolitis 15809 Necrotising enterocolitis – Confirmed is recorded		
	NEC onset PMA	Postmenstrual age for NEC onset		
NEC related variables (raw data)	PostmortemConfirmation	If a necrotising enterocolitis diagnosis was made at any point at admission, specify if the post mortem confirmed it.	N No Y Yes 9 Unknown	
	SurgicalProcedure	Surgical procedure on the date and time specified OPCS coded and/or SNOMED CT		
	StomaInSitu	N No Y Yes		
	InvestigateAbdsigns	N No Y Yes		
	XrayAppearance	?? 1 Pneumatosis 2 Air in the liver 3 Pneumoperitoneum 4 Fixed loop 5 Gasless 9 None of the above		
	abdominalxrayfindings	01 Abdominal distension 02 Abdominal tenderness 03 Increased/ bilious aspirates 04 Abdominal discolouration 05 Abdominal mass 06 Bloody stools		

		07 Mucousy stools 09 None of the above		
	TransferredOutManagementNEC	N No Y Yes		
	necLaparotomy	0 Laparotomy not required 1 Laparotomy required but PATIENT too ill to carry it out 2 Laparotomy required and carried out		
	laparotomyConfirm	N No Y Yes		
	necHistologyConfirmed	0 Not confirmed 1 Yes confirmed 9 No histological inspection/Not applicable		
Infant: Sepsis suspected on first day	SuspectedSepsisFirstDays	Where the following is true: DayDateAnon in first day of life AND first full day in unit AND SuspectedSepsis >= 1 (Y)	0 = No 1 = Yes 9 = Unknown	
Early onset blood stream infection		Defined from Infection Cultures (Episodic) recorded in the first 3 days/before day 3 • Pure growth of pathogen from blood OR • Pure growth of pathogen from CSF OR • Either a pure growth of a skin commensal or a mixed growth with ≥3 clinical signs at the time of blood sampling		
Late onset blood stream infection NNAP definition		Defined from Infection Cultures (Episodic) recorded after day 3 • Pure growth of pathogen from blood OR • Pure growth of pathogen from CSF OR • Either a pure growth of a skin commensal or a mixed growth with ≥3 clinical signs at the time of blood sampling	Dichotomous (No infection=0, Infection=1) Dichotomous Unknown = 9	
Late onset infection, non-NNAP		5 consecutive days of antibiotic treatment defined as 5 consecutive days of any of the following (including in combination and changing during the 5 days) after day 3 Daily care medication •1010155 Benzyl Penicillin •1010158 Augmentin •1010179 Flucloxicillin •500012 Flucloxacillin •500016 Gentamicin •500072 Co-amoxiclav •500086 Co-amoxiclav •500084 Ciprofloxacin	Dichotomous (No infection=0, Infection=1) Dichotomous	

		•500029 Netilmicin •500002 Amikacin •500211 Tazocin •500023 Metronidazole •500040 Vancomycin •500007 Cefotaxime •500004 Ampicillin •500009 Cefuroxime •500008 Ceftazidime •500175 Ceftriaxone •500032 Piperacillin •500206 Oflacillin •500005 Azlocillin •1010171 Linezolid •1010271 Cefalexin •1010139 Amoxicillin •500070 Amoxicillin •500128 Meropenem •500118 Imepenem •500145 Imipenem •500069 Ambisome (Liposomal Amphotericin) •500003 Amphotericin •1010195 Amphotericin Liposoma		
Early onset infection (pragmatic)		>5 consecutive days of antibiotic treatment defined as 6 consecutive days of any of the following (including in combination and changing during the 6 days) before day 3 Daily care medication		
Time to reach full feeding		First day of 3 consecutive days where parenteral nutrition or intravenous fluid are not recorded		

Covariates/confounders

BadgerID	AnonPatientID		N/A	Episode-specific identifier for each baby
Gestational age at birth	GestationWeeks		Numeric (10 - 49)	
	GestationDays		Numeric (0-6) 9 = Unknown	
	GA total days	Weeks x 7 + days		

Birth weight	Birthweight		Numeric (g) 99999 = Unknown	Accepted range: 001 – 9998g
Birth head circumference	BirthHeadCircumference	Can perhaps be useful to differentiate between symetrical and asymetrical IUGR		
Sex	Gender		0 = Unknown 1 = Male 2 = Female 9 = Not specified	
Birth weight z-score		Specify Marsal or Fenton or WHO	Numeric 99999 = Unknown	
Ethnicity	Race	Combine parental ethnicities (see row 36,39) Parents Demographics ETHNIC CATEGORY (MOTHER) (categorical) Coded as: WHITE (A - British, B - Irish, C - Any other white background); MIXED (D - White and Black Caribbean. E - White and Black African, F - White and Asian, G - Any other mixed background); ASIAN OR ASIAN BRITISH (H - Indian, J - Pakistani, K - Bangladeshi, L - Any other Asian Background); BLACK OR BLACK BRITISH (M - Caribbean, N - African, P - Any other Black background); OTHER ETHNIC GROUPS (R - Chinese, S - Any other ethnic group); UNKNOWN (Z, DTA - Not stated, 99 - Not known) This data item is based on self-reported ethnicity as recorded in maternity notes	?e.g. Categorised into four groups (White=1; Asian & Mixed=2; Black & Mixed=3; Other and not given=4)	
Smoking during pregnancy	Smoking	Pregnancy Details MOTHER CURRENT SMOKER AT BOOKING INDICATOR (categorical, codes 1-6)	0 = No 1 = Yes 9 = Unknown	
Multiplicity	FetusNumber	N/A	Numeric 99 = Unknown	
Birth year	BirthYear	N/A	Numeric 9999 = Unknown	
Birth year (mother)	BirthYearMother	N/A	Numeric 9999 = Unknown	
Parity of mother	Primiparity	Pregnancy Details PREGNANCY TOTAL PREVIOUS PREGNANCIES Dichotomous: code 00=Y; code 01- 29=N	Primiparous: Dichotomous (Not first pregnancy=0; First pregnancy=1)	
Maternal deprivation score	PostCodeMother	LSOA centiles		
	MumEducation			
	MumOccupation			
Maternal Diabetes (Y/N)	MaternalDiabetes	Where the following is true: ProblemsMedicalMother = 15 THEN (1) ProblemsMedicalMother = 00 THEN (0)	0 = No maternal diabetes 1 = Maternal Diabetes Present 99 = Unknown	Blank entries coded in as 99

Maternal gestational diabetes (Y/N)	MaternalGestDiabetes	Where the following is true: ProblemsDuringPregnancy = 33 THEN (1) ProblemsDuringPregnancy = 00 THEN (0)	0 = No gestational diabetes 1 = Gestational diabetes present 99 = Unknown	Blank entries coded in as 99
Maternal pre-eclampsia requiring pre-term birth (Y/N) Maternal pre-eclampsia	PreEclampsia	Where the following is true: ProblemsDuringPregnancy = 31 THEN (1)	0 = No pre-eclampsia 1 = Pre-eclampsia present 99 = Unknown	Blank entries coded in as 99
Maternal gestational hypertension (Y/N)	MaternalGestHypTension	Where the following is true: ProblemsDuringPregnancy = 30 THEN (1)	0 = No gestational hypertension 1 = Gestational hypertension present 99 = Unknown	Blank entries coded in as 99
Maternal prolonged rupture of membranes	ROMTimeAnon	Derived (Minutes)	Numeric	Number of minutes from birth to event
	Prolonged_ROM	Where the following is true: ProblemsDuringPregnancy = 20 THEN (1)	0 = No prolonged rupture 1 = Prolonged rupture present	
Maternal suspected chorioamnionitis (Y/N)	Chorioamnionitis	Where the following is true: MaternalPyrexiaInLabour38c = 1 OR IntrapartumAntibioticsGiven = 1 THEN (1)	0 = No 1 = Yes 9 = Unknown	Blank entries coded in as 9
Intrapartum Antibiotics	IntrapartumAntibioticsGiven	IntrapartumAntibioticsGiven = 1 THEN (1)	0 = No 1 = Yes 9 = Unknown	
Maternal pyrexia	MaternalPyrexiaInLabour	MaternalPyrexiaInLabour38c = 1 THEN (1)	0 = No 1 = Yes 9 = Unknown	
Maternal GBS	MaternalGBS	ProblemsInfctPregnancyMother = Group B streptococcus THEN (1)	0 = No 1 = Yes 9 = Unknown	
Maternal receipt of antenatal steroids (Y/N)	MaternalAntenatalSteroids	Where the following is true: SteroidsAntenatalGiven = 1 AND SteroidsAntenatalCourses = 1 THEN (1) SteroidsAntenatalGiven = 1 AND SteroidsAntenatalCourses = 2 THEN (2) SteroidsAntenatalGiven = 0 AND SteroidsAntenatalCourses = 0 THEN (0)	0 = None given 1 = Complete 2 = Incomplete 9 = Unknown	
Maternal receipt of antenatal magnesium sulphate (Y/N)	MagnesiumSulphate	N/A	0 = No 1 = Yes 9 = Unknown	

Delivery mode	ModeOfDelivery	Emergency caesarean = 1 Elective caesarean = 2 Vaginal spontanous = 3	1 = emergency 2 = elective 3 = vaginal 9 = unknown	
Induced delivery	Onsetoflabour	Spontanous = 1 Induced (medical and/or surgical) = 2 None (i.e. caesarean) = 3	1 = spontanous 2 = Induced 3 = None 9 = unknown	
Labour before caesarean	ModeofDelivery Caesarean	Yes No	0 = no labour 1 = labour	
Delivery categories	Delivery categories	Categorize above: Vaginal AND Spontanous = 1 Vaginal AND Induced = 2 Emergency caesarean AND labour = 3 Emergency caesarean AND nolabour = 4 Elective caesarean AND labour = 5 Elective caesarean AND nolabour = 6	1 = VagSpon 2 = VagInduc 3 = EmergCaesLab 4 = EmergCaesNolab 5 = ElectCaesLab 6 = ElectCaesNolab 9 = unknown	
Infant Apgar score at 1 minutes	apgar_1min	N/A	0-10 Apgar score 99 = Unknown	
Infant Apgar score at 5 minutes	apgar_5min	N/A	0-10 Apgar score 99 = Unknown	
Infant Apgar score at 10 minutes	apgar_10min	N/A	0-10 Apgar score 99 = Unknown	
Spontaneous respiration time of onset	SpontaneousRespirationTime	1 <1 mins 2 1-1.5 mins 3 1.6-2 mins 4 2.1-3 mins 5 3.1-4 mins 6 4.1-5 mins 7 > 5mins		
Infant: chest compressions administered (Y/N)	CardiacMassage	Where the following is true: MethodsOfResuscitation = 16 THEN (1)	0 = No cardiac massage 1 = Cardiac massage 99 = Unknown	
Infant: Intubation at delivery (Y/N)	IntubationDelivery	Where the following is true: MethodsOfResuscitation = 15 THEN (1)	0 = No intubation 1 = Intubation 99 = Unknown	
Infant: Ventilation at delivery (Y/N)	VentilationDelivery	Where the following is true: MethodsOfResuscitation = 14 THEN (1)	0 = No IPPV 1 = IPPV 99 = Unknown	
Infant: Emergency resuscitation drugs administered (Y/N)	ResusDrugsAdmin	Where the following is true: MethodsOfResuscitation = 17 OR MethodsOfResuscitation = 88 THEN (1)	0 = No resuscitation drugs administered	

			1 = Resuscitation drugs administered 99 = Unknown	
Infant: Surfactant administered (Y/N)	SurfactantGivenResuscitation	N/A	0 = No 1 = Yes 9 = Unknown	
Infant: Umbilical cord pH	CordPhArterial	N/A	6.00-8.00 9.99 = Unknown	
	CordVenousPH	N/A	6.00-8.00 9.99 = Unknown	
Umbilical cord lactate	CordlLactate			
Umbilical cord base excess	CordBE	Labour and Delivery Details UMBILICAL CORD BLOOD BASE EXCESS CONCENTRATION (ARTERIAL) Continuous OR if not available use Labour and Delivery Details UMBILICAL CORD BLOOD BASE EXCESS CONCENTRATION (VENOUS)	CordBaseExcess: Continuous (to 1 decimal place) CordBaseExcessMs: Binary missing indicator created (Not missing=0; Missing=1)	
Base excess 12h worst	WorstBaseWithin12			
Blood transfusion day 1-2	BloodProductsTrans	On day 1 and day 2 Daily care blood transfusion BLOOD TRANSFUSION PRODUCT TYPE	1 = yes 0 = no	
Umbilical catheters	LinesIn	Only on day 1-2 (admission) 1 Peripheral arterial line 2 Umbilical arterial line if THEN YES 3 Umbilical venous line if THEN YES 4 Percutaneous central venous line (long line) 5 Surgically inserted central venous line 6 Peripheral venous line 9 Not Applicable/ No Lines in Situ	1 = yes 0 = no 9 = unknown	
Birth place	PlaceofBirthNHSCode		Organization code	
Time to admission		Admission Details CRITICAL CARE START YEAR AND MONTH and NUMBER OF MINUTES (BIRTH TO EVENT)		
Assisted Ventilation		On day 1-2		
Surfactant therapy		On day 1-2		
Feeding advancement (early feeding)		198:9 E.g. any of the following items entered in the Daily Care Fluids and Feeding during the first 3 days • Any entry (1-6) under ENTERAL FEED TYPE GIVEN	Dichotomous (No enteral feeds=0; provided enteral feeds=1)	

		• OR any entry (0-88) under FORMULA MILK OR MILK FORTIFIER TYPE • OR any value >0 for TOTAL VOLUME OF MILK RECEIVED • OR any entry (1-8) under ENTERAL FEEDING METHOD NO ENTERAL FEEDING GROUP DEFINED AS All other babies not fulfilling above criteria		
Feeding		On day 1-2 1 Suckling at the breast 2 Mother's fresh expressed breast milk 3 Mother's frozen expressed breast milk 4 Donor expressed breast milk 5 Breast milk fortifier 6 Formula 9 Not applicable (Nil by mouth)	1 = Formula 2 = Human milk 3 = Mix ? 0 = nil 9 = unknown	
Probiotics		On day 1-2	1 = yes 0 = no	
Fluids and feeding: Parenteral nutrition today (partial or total)	ParenteralNutrition	PN on day1-2	1= yes 0 = no	
Unit of first admission	ProviderNHSCode	N/A	xxxxx - NHS organisational code ZZ210 - non-NHS England and Wales organisation (private/N.Ireland/Scotland) ZZ203 – Not known ZZ999 - Missing	
Infant: Admission temperature	FirstAdmitTemperature	N/A	24-45 77.7 = Not Recordable	Measurement at first admission
Infant: Admission mean blood pressure	FirstAdmissionBP	N/A	10-150 999 = Unknown	Measurement at first admission
Infant: Admission blood glucose	FirstAdmissionBloodGlucose	N/A	0.0-50.0 99.9 = Unknown	Measurement at first admission
Infant: Admission heart rate	FirstAdmissionHR	N/A	50-350 999 = Unknown	Measurement at first admission
Infant: Admission oxygen saturation	FirstAdmissionOxygenSaturation	N/A	10-100 999 = Unknown	Measurement at first admission

Infant: Surfactant administered on first day (Y/N)	SurfactantGivenFirstDays	Where the following is true: DayDateAnon in first day of life AND first full day in unit AND SurfactantGiven = 1 THEN (Y)	0 = No 1 = Yes 9 = Unknown	
Infant: Mechanical ventilation on first day (Y/N)	RespiratorySupportFirstDays	Where the following is true: DayDateAnon in first day of life AND first full day in unit AND RespiratorySupport = 1 OR AddedO2 = 11 OR VentilationMode >= 1 THEN (1)	0 = No mechanical ventilation on first day 1 = Mechanical ventilation on first day 9 = Unknown	Blank entries coded as 9
Infant: Inotropes administered on first day (Y/N)	InotropesFirstDays	Where the following is true: DayDateAnon in first day of life AND first full day in unit AND DailyDrugs in 500098 Dopamine 500096 Dobutamine 500056 Adrenaline 500210 Noradrenaline 500116 Hydrocortisone 1010173 Milrinone OR InotropesGiven = 1 THEN (Y)	0 = No 1 = Yes, Inotropes given today 9 = Unknown	Blank entries coded in as 9
Hemodynamically significant PDA	PDA	Treated on day 1-2 Yes/No		
	Cardiovascular: Treatment for patent ductus arteriosus (PDA)	1 Indometacin/Indomethacin 2 Ibuprofen 3 Surgery 9 Not applicable		
Neurology: Central tone	Centraltone	At admission (On day 1-2) 1 Normal 2 Increased 3 Decreased	0 = normal 1 = abnormal incl floppy	
Admission: Time of cord clamping	CordClamp	Cord clamped immediately after birth	0 = No 1= Yes 9 = Unknown	
Infant: Transfer on first day (Y/N)	TransferOnFirstDay	Where the following is true: AdmitTimeAnon <= 1440 AND DischTimeAnon <= 1440 AND DischargeDestination does not equal 3 AND ProviderCode is different from POBCode AND EpisodeNumberBaby = 2 THEN (1)	0 = No 1 = Yes 9 = Unknown	

		Same as: Admission Details SITE CODE (OF ADMITTING NEONATAL UNIT) or ORGANISATION CODE (OF ADMITTING NEONATAL UNIT) Different from Baby Demographics SITE CODE (OF ACTUAL PLACE OF DELIVERY) or ORGANISATION CODE (OF ACTUAL PLACE OF DELIVERY) And Baby Demographics EPISODE NUMBER		
	TransferDestination	Derived from ProviderNHSCode	xxxxx - NHS organisational code ZZ210 - non-NHS England and Wales organisation (private/N.Ireland/Scotland) ZZ203 – Not known ZZ999 - Missing	
Level of initial neonatal unit	POBLevel	Derived from POBNHSCode	0 = Non-NNU 1 = SCU 2 = LNU 3 = NICU	
Neonatal network	POBNetwork	Derived from POBNHSCode	Text	

Additional outcomes

Brain injury on imaging	BrainInjuryImaging	Where the following is true: LeftIVH OR RightIVH >= 3 OR PVL = Y THEN (1)	0 = No brain injury on imaging 1 = Brain injury on imaging 9 = Unknown	Blank entries coded as 9
Treated ROP	Treated_ROP	Where the following is true: ROPSurgery = 1 OR RightEyeSurgery = 1,2,3,4 OR LeftEyeSurgery = 1,2,3,4	0 = No treatment 1 = Treatment given	
Maximum stage of ROP	Max_ROP	N/A	0 – No ROP 1 – Stage 1 ROP 2 - Stage 2 ROP 3 – Stage 3 ROP 4 – Stage 4 ROP 5 – Stage 5 ROP A – Aggressive posterior ROP	The maximum stage between right and left eye

Bronchopulmonary dysplasia	BPD	Where the following is true: CGA = 36 calculated from GestationWeeks and GestationDays AND RespiratorySupport = 1 OR VentilationMode >= 1 OR Added02 > 0 THEN (1)	0 = No respiratory support given at 36 weeks 1 = Respiratory support at 36 weeks 9 = Unknown	Blank entries coded as 9
Need for surgical procedures (possible more than one)	SurgicalProcedures	Where the following is true: PrincipleProceduresDuringStay = 100033 Surgery for meconium ileus (von) 100076 Skin or soft tissue surgery requiring general or spinal anaesthesia (Description Required) 11222 Closure of small intestine/ileal perforation 11501 Laparoscopy 11904 Colostomy 11905 Ileostomy 1010826 Major surgery OR MajorSurgeryToday = Y THEN (1)	0 = No 1 = Yes 9 = Unknown	Blank entries coded as 9
Seizures	Seizures	Where the following is true: PrincipalDiagnosisAtDischarge = 10957 Seizures 15192 Seizure disorder 15194 Seizure disorder (cause unknown) 15195 Status epilepticus 15848 Seizures OR Convulsions = 1 THEN (1)	0 = None 1 = Seizures at discharge 9 = Unknown	Blank entries coded as 9
Weight at 36 weeks corrected gestational age (CGA)	CGA_weight	Where the following is true: DayWorkingWeight at 36 weeks (+/- 3 days) CGA	Numeric (g) 99999 = Unknown	Accepted range: 001 – 9998g
Exact CGA on day of measurement	CGA_exact	Calculated from DayDateAnon and GestationWeeks and GestationDays	Numeric	
Weight at discharge	DischWeightFinalEps	DayWorkingWeight on the last day of the last episode	Numeric (g) 99999 = Unknown	Accepted range: 001 – 9998g
Weight SDS at discharge		Defined as the following data item on the final day of neonatal care: • Daily Care General Information PERSON WEIGHT IN GRAMS If final day is not entered, the penultimate day is used	Continuous	
Head circumference at discharge	DischHeadCircumFinalEps	DayHeadCirc on the last day of the last episode	Numeric (cm) 99.9 = Unknown	
CGA day of discharge	CGA_DischFinalEps	Calculated from DayDateAnon and GestationWeeks of the last episode	Numeric	

Blindness	Vision_impairment	Where the following is true: Vision_visual_problems = 1 OR vision_defect_not_correctable = 1 OR Vision_blind = 1 THEN (1)	0 = No 1 = Yes 9 = Unknown	
Deafness	auditory_hearing_impairment	N/A	0 = No 1 = Yes 9 = Unknown	
Ability to walk	neuromotor_unable_walk_without_a	N/A	0 = No 1 = Yes 9 = Unknown	
Exclusions for congenital gastrointestinal malformations	Exclusions_Malformations	Where the following is true: Diagnosis table code in eTable1 THEN (1)	0 = No, none of the listed malformations present 1 = Yes	
Exclusions for life-limiting conditions or conditions requiring surgery	Exclusions_LifeLimitingOrSurgery	Where the following is true: Diagnosis table code in eTable2 THEN (1)	0 = No, none of the conditions present 1 = Yes	
Clinical diagnoses at discharge	DiagnosesAtDischarge	ICD-10 and/or SNOMED CT Can be derived from daily records. [Secondary/exploratory outcomes]	For each selected outcome 0=No 1=Yes	

Appendix 2. DAG code for dagitty.net (for direct insertion on website browser platform)

```
dag {
bb="0,0,1,1"
"Abruptio/bleeding" [latent,pos="0.146,0.569"]
"Anemia/Transfusion" [pos="0.304,0.710"]
"Antenatal steroids" [pos="0.571,0.355"]
"Assisted ventilation" [pos="0.372,0.734"]
"Clinical signs" [adjusted,pos="0.395,0.643"]
"Delivery mode and C-section type" [adjusted,pos="0.488,0.294"]
"Feeding initiation" [pos="0.544,0.773"]
"Fetal flow" [latent,pos="0.276,0.465"]
"Formula feeding" [pos="0.469,0.788"]
"Maternal antibiotics " [pos="0.491,0.203"]
"Maternal infection" [adjusted,pos="0.148,0.457"]
"Probiotic initiation" [pos="0.625,0.754"]
"Site/outborn" [adjusted,pos="0.516,0.648"]
"Surfactant therapy" [pos="0.367,0.806"]
"Umbilical catheters" [pos="0.241,0.736"]
AB [exposure,pos="0.510,0.486"]
BW [adjusted,pos="0.434,0.486"]
Dyscolonization [latent,pos="0.556,0.261"]
Ethnicity [pos="0.252,0.204"]
GA [adjusted,pos="0.409,0.385"]
GDM [pos="0.386,0.068"]
IUGR [adjusted,pos="0.361,0.484"]
Ischemia [latent,pos="0.304,0.632"]
Multiplicity [pos="0.311,0.209"]
NEC [outcome,pos="0.609,0.483"]
PDA [pos="0.217,0.667"]
Parity [pos="0.290,0.143"]
Preeclampsia [pos="0.413,0.250"]
SES [pos="0.309,0.047"]
Sepsis [pos="0.227,0.567"]
Sex [pos="0.239,0.272"]
Smoking [pos="0.337,0.097"]
"Abruptio/bleeding" -> "Anemia/Transfusion" [pos="0.243,0.615"]
"Abruptio/bleeding" -> Ischemia
"Anemia/Transfusion" -> Ischemia
"Anemia/Transfusion" -> NEC
"Antenatal steroids" -> "Clinical signs" [pos="0.489,0.410"]
"Antenatal steroids" -> NEC
"Assisted ventilation" -> "Surfactant therapy"
"Assisted ventilation" -> NEC
"Clinical signs" -> "Assisted ventilation"
"Clinical signs" -> AB [pos="0.386,0.578"]
"Clinical signs" -> NEC [pos="0.423,0.577"]
"Delivery mode and C-section type" -> "Antenatal steroids"
"Delivery mode and C-section type" -> AB
"Delivery mode and C-section type" -> Dyscolonization
"Delivery mode and C-section type" -> GA
"Delivery mode and C-section type" -> NEC
"Feeding initiation" -> NEC [pos="0.601,0.675"]
```

Appendix 2. DAG code for dagitty.net (for direct insertion on website browser platform)

```
"Fetal flow" -> "Delivery mode and C-section type"
[pos="0.261,0.334"]
"Fetal flow" -> IUGR
"Fetal flow" -> NEC [pos="0.385,0.603"]
"Formula feeding" -> NEC [pos="0.555,0.719"]
"Maternal antibiotics " -> Dyscolonization
"Maternal antibiotics " -> NEC [pos="0.642,0.236"]
"Maternal infection" -> "Clinical signs" [pos="0.362,0.575"]
"Maternal infection" -> "Delivery mode and C-section type"
[pos="0.238,0.289"]
"Maternal infection" -> "Maternal antibiotics " [pos="0.229,0.267"]
"Maternal infection" -> AB [pos="0.322,0.356"]
"Maternal infection" -> NEC [pos="0.575,0.705"]
"Maternal infection" -> Sepsis
"Probiotic initiation" -> NEC [pos="0.639,0.652"]
"Site/outborn" -> "Clinical signs"
"Site/outborn" -> "Feeding initiation"
"Site/outborn" -> "Formula feeding"
"Site/outborn" -> "Probiotic initiation"
"Site/outborn" -> AB
"Site/outborn" -> NEC
"Surfactant therapy" -> NEC
"Umbilical catheters" -> Ischemia
AB -> NEC
BW -> AB
BW -> NEC [pos="0.526,0.569"]
Dyscolonization -> NEC [pos="0.604,0.306"]
Ethnicity -> NEC [pos="0.720,0.028"]
GA -> "Antenatal steroids"
GA -> AB
GA -> BW
GA -> NEC
GDM -> NEC [pos="0.806,0.106"]
IUGR -> "Delivery mode and C-section type" [pos="0.319,0.358"]
IUGR -> BW
IUGR -> NEC [pos="0.512,0.614"]
Ischemia -> "Clinical signs"
Ischemia -> NEC
Multiplicity -> NEC [pos="0.697,0.057"]
PDA -> Ischemia
Parity -> NEC [pos="0.756,0.077"]
Preeclampsia -> "Delivery mode and C-section type"
SES -> GDM
SES -> NEC [pos="0.852,0.023"]
SES -> Smoking
Sepsis -> "Clinical signs" [pos="0.346,0.586"]
Sepsis -> Ischemia
Sepsis -> NEC [pos="0.478,0.596"]
Sex -> NEC [pos="0.690,0.026"]
Smoking -> NEC [pos="0.780,0.096"]
}
```

BMJ Open

Early antibiotic use and incidence of necrotising enterocolitis in very preterm infants: a protocol for a UK based observational study using routinely recorded data

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Early antibiotic use and incidence of necrotising enterocolitis in very preterm infants: a protocol for a UK based observational study using routinely recorded data

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ABSTRACT

Introduction

Necrotising enterocolitis (NEC) remains a major contributor to preterm mortality and morbidity. Prolonged duration of antibiotic therapy after delivery is associated with later NEC development but recent evidence suggests that absence of antibiotic treatment after delivery may also increase NEC risk. We will explore this controversy using a large pre-existing dataset of preterm infants in the United Kingdom (UK).

Methods and analysis

This is a retrospective cohort study using data from UK National Neonatal Research Database (NNRD) for infants born 01/01/2012 to 31/12/2020. Eligible infants will be <32 weeks gestation, alive on day three. Primary outcome is development of severe NEC, compared in infants receiving early antibiotics (days 1-2 after birth) and those not. Subgroup analysis on duration of early antibiotic exposure will also occur. Secondary outcomes are: late onset sepsis (LOS), total antibiotic use, pre-discharge mortality, retinopathy of prematurity (ROP), intraventricular haemorrhage (IVH), bronchopulmonary dysplasia (BPD), focal intestinal perforation and any abdominal surgery. To address competing risks, incidence of death before day 7, 14 and 28 will be analysed. We will perform logistic regression and propensity score matched analyses. Statistical analyses will be guided by NEC risk factors, exposures and outcome presented in a causal diagram. These covariates include but are not limited to gestational age, birth weight, small for gestational age (SGA), sex, ethnicity, delivery mode, delivery without labour, Apgar score, early feeding and probiotic use. Sensitivity analyses of alternate NEC definitions, specific antibiotics and time of initiation will occur.

Ethics and dissemination

We will use de-identified data from NNRD, which holds permissions for the original data, from which parents can opt out and seek study- specific Research Ethics approval. The results will help to determine optimal use of early antibiotics for very preterm infants.

Implications

This data will help optimise early antibiotic use in preterm infants.

ARTICLE SUMMARY

Strengths and limitations of the study

Strengths:

- Use of the NNRD gives access to a very large dataset of preterm infants
- The primary outcome (NEC) and the many contributory covariates are routinely recorded in this dataset
- Analysis by both regression and using propensity matching optimises learning from this large dataset

Limitations:

- Data entry may not always be as accurate as that collected specifically within a trial
- The diagnosis of NEC has no gold standard to allow standardisation across units

INTRODUCTION

Around 3% of all babies are born very preterm (VPT, <32 weeks' gestation) and they require prolonged hospital stay, commonly including intensive care. Survival in these VPT infants (VPTI) has increased dramatically in recent years, but death is still common (~10% overall) as are life-long physical and cognitive impairment.[1] In the UK around 10,000 VPTI are born every year, representing an annual cost to the NHS of ~£3 billion.[2] The commonest cause of death or serious illness in preterm infants after the first few days are gut or infectious complications such as necrotising enterocolitis (NEC) or late onset sepsis (LOS).[3] Although knowledge around NEC, and preventive practices such as use of mothers own milk (MOM), donor human milk (DHM) and probiotics are increasing, there has been little reduction in NEC incidence over recent years[4,5], and mechanisms underlying the development of NEC are poorly understood. Antibiotic use as part of neonatal intensive care is common, particularly immediately after birth when infection is implicated in preterm delivery – studies show more than half of infants weighing <1000g routinely received more than 5 days antibiotics at birth[6]. Antibiotic use in VPTI has been implicated in NEC development in several ways. Studies show an increase in NEC incidence with increased duration of empirical early antibiotics[7,8] and alteration of the gut microbiotia (dysbiosis) has been mechanistically linked to NEC development [9]. However recent observational data from 13 NICUs from 5 continents (n=2831) identified that NEC incidence was higher in infants who did not receive empirical antibiotics early after birth, despite higher gestational age, compared to those receiving them (OR: 1.8 (95% CI 1.1-2.9), with even higher odds ratio when adjusted for relevant confounders (OR: 4.0 (95% CI 2.1-7.3)) [10]. In contrast, results from a very recent study in preterm infants with low risk of infection shows opposite trends of lower odds in those not treated, but is underpowered for NEC as outcome (n=641, OR:0.7 (95%CI 0.3-1.5))[11]. There is increasing focus on antibiotic stewardship, and it can be

expected that the proportion of infants that are not given antibiotics after preterm birth will increase in the coming years. Therefore, it is important to know if lower early usage of antibiotics will increase the incidence of NEC. While there have been calls for a trial of routine early antibiotic treatment[12] in very preterm babies, and a single trial has so far attempted to do this, there are important logistical difficulties[13] with such an approach. The National Neonatal Research Database (NNRD) provides a large, population level dataset that can be used to further test the hypothesis that early empiric antibiotic treatment reduces the incidence of NEC in preterm infants, and allows adjustment for confounding through the large number of patient level covariates recorded in the NNRD.

METHODS AND ANALYSIS

Design

Retrospective cohort study using routinely recorded clinical data held in the NNRD.

Data source

NNRD holds data from all infants admitted to National Health Service (NHS) neonatal units in England and Wales from around 90,000 infants annually. Neonatal units in England and Wales have contributed data since 2012. Data are entered by contributing units to a point-of-care electronic dataset and a defined dataset is extracted by NNRD. Data is extracted quarterly and sent to the Neonatal Data Analysis Unit (NDAU), based at Imperial College, London [14]. The data includes variables pertinent to the present analysis, including demographics, exposure and outcome variables.

Eligibility criteria

Eligible infants must have been born at <32 weeks gestation, be cared for in a unit contributing data to NNRD, and be alive at day 3. Infants will be excluded if they have a known severe congenital or gastro-intestinal anomaly (excluding the presence of a patent ductus arteriosus, eTable 1 and 2) or have had abdominal surgery before day 3.

Time period

Infants born between 01/01/2012 and 31/12/2020 will be included.

Setting

UK neonatal units in England and Wales contributing to NNRD.

Definitions

Exposure (primary)

Receipt of any intravenous antibiotic drug (Appendix 1) for any of the first 2 days after birth.

Comparator: Did not receive any antibiotics for any of the first 2 days after birth.

Primary outcomes

Severe NEC resulting in death or surgery as defined by Battersby[4].

Secondary outcomes

Secondary outcomes for analysis are the effects of early antibiotic exposure on

1. Late onset sepsis (blood stream or cerebrospinal fluid (CSF) confirmed pure growth in culture (National Neonatal Audit Programme (NNAP) definition) after first 3 days and/or treatment with 5 days of antibiotics and a concurrent diagnosis of infection after the first 3 days)
2. Total antibiotic use (number of days with any treatment of antibiotics during admission)
3. Length of stay (postnatal age at discharge or death)
4. Time to reach full feeding (first day of 3 consecutive days where parenteral nutrition or intravenous fluid are not recorded
5. Growth (change in standard deviation score between birth and 36 weeks and discharge)

Further, we will analyse effects on some relevant adverse outcomes:

6. Total pre-discharge mortality
7. Death prior to day 7, day 14, day 28
8. Bronchopulmonary dysplasia (respiratory support given at 36 weeks)
9. Retinopathy of prematurity (ROP) (received treatment for ROP, according to NNRD definition)
10. Brain injury (Intraventricular haemorrhage grade 3 or above or cystic leucomalacia diagnoses recorded)
11. Need for surgical procedures (Appendix 1)

Comparison of different durations of early antibiotic exposure will be performed based on the following categories:

- Antibiotic duration no longer than 3 days
- Antibiotic duration 3-5 days
- Antibiotic duration longer than 5 days without positive culture (blood stream or CSF confirmed pure growth in culture (NNAP definition) in the first 3 days.

For the above analyses, infants with a positive blood or CSF culture in the first 3 days will be excluded.

A specific subgroup of interest are the infants that are considered to have low risk of early onset sepsis (EOS), specified as fulfilling all of the following prenatal characteristics: no premature rupture of membranes (PROM), no labour and no (suspected) chorioamnionitis.

Additional subgroup analyses will be performed for infants with gestation age <28 weeks and birth weight <1000g.

Sample size

Observed NEC incidence noted in a previous study on a total 2831 infants from five different continents, using criteria for NEC diagnosis in keeping with pragmatically defined NEC, was 9% when early antibiotic treatment was absent and 4% when antibiotic was provided in the

first three days[10]. We hypothesise to find a similar antibiotic related proportional reduction in incidence of severe NEC in this study, based on data collected over nine years (2012-2020) from around 45,000 infants. In an earlier report based on a NNRD subgroup, the incidence of severe NEC was 3.2% for infants born <32 weeks[4]. The cohort event estimate is 1440 cases.

Data required

Appendix 1 carries the full list of variables considered relevant for extraction from NNRD including definitions of constructed items/variables.

Potential confounders

Several covariates are relevant to include in the analysis as potential confounders. We will take a hypothesis driven approach to the selection of covariates. A causal diagram (Directed Acyclic Graphs, DAG, Figure 1) is drawn and analysed with relevant variables and potential confounders related to antibiotic exposure and NEC outcome. Nodes and edges are determined based on literature and subject matter knowledge. The selected covariates are considered to reflect conditions prior to the defined exposure (i.e. within day 1-2 after birth). For several variables, only proxies will be available (Table 1).

Table 1 Overview of variables

Variable	Class/type	Expected availability in NNRD	Importance for effect estimation
AB	Exposure	Available (definable)	
NEC	Outcome	Available (definable)	
Site	Confounder	Available	Minimal sufficient adjustment set to model the direct and total effect of AB on NEC according to proposed DAG
BW	Confounder	Available	
Delivery mode[1]	Confounder	Available (categories)	
Clinical signs[2]	Confounder	Available (proxies)	
Maternal infection	Confounder	Available (clinical)	
GA	Confounder	Available	
IUGR	Confounder	Available (definable)	
Fetal flow	Ancestor of Exp & Out	Unobserved	Blocked by IUGR and delivery mode

	(indirect)		
Ischemia	Ancestor of Exp & Out (indirect)	Unobserved	Blocked by Clinical Signs
Preeclampsia	Ancestor of Exp & Out (indirect)	Available	Blocked by delivery mode
Antenatal steroids	Ancestor of Outcome	Available	Blocked by clinical signs
Sepsis	Ancestor of Exp & Out (indirect)	Available (definable/proxy)	Blocked by clinical signs
PDA	Ancestor of Exp & Out (indirect)	Available (definable)	Blocked by clinical signs
Umbilical catheters	Ancestor of Exp & Out (indirect)	Available	Blocked by clinical signs
Anaemia/transfusion	Ancestor of Exp & Out (indirect)	Available (proxy, i.e. transfusions)	Blocked by clinical signs
Sex	Ancestor of Outcome	Available	Precision variable
Ethnicity	Ancestor of Outcome	Available	Precision variable
Multiparity	Ancestor of Outcome	Available	Precision variable
Smoking	Ancestor of Outcome	Available	Precision variable
GDM	Ancestor of Outcome	Available	Precision variable
Socioeconomic status	Ancestor of Outcome	Available (proxy i.e. deprivation score)	Precision variable
Maternal antibiotics	Ancestor of Outcome	Available (intrapartum)	Precision variable
Dyscolonisation	Ancestor of Outcome	Unobserved	Precision variable
Assisted ventilation	Ancestor of Outcome	Available	Precision variable
Surfactant therapy	Ancestor of Outcome	Available	Precision variable
Formula feeding	Ancestor of Outcome	Available	Precision variable
Feeding initiation	Ancestor of Outcome	Available	Precision variable
Probiotic initiation	Ancestor of Outcome	Available	Precision variable

[1]Specification of different clinical conditions with important impact on decision to treat with AB, categorized as: Vaginal AND Spontaneous, Vaginal AND Induced, Emergency caesarean AND labour, Emergency caesarean AND no labour, Elective caesarean AND labour, Elective caesarean AND no labour.
[2]Respiratory/circulatory/unspecific signs/symptoms/parameters used clinical assessment and decision making related to decision to treat with antibiotics

STATISTICAL ANALYSES

Primary analyses

Previous work using logistic regression included the following covariates in the model for the hypothesis: NICU (random effect) + GA + birthweight+ sex+ delivery mode + APGAR scores + antenatal steroids + feeding type. We aim to test the hypothesis with data from NNRD using the same regression model as used in the previous work (variables 1-8 in Table 2) and also an expanded regression model with inclusion of all potentially relevant variables (Table 2). Results will be presented as adjusted odds ratios with 97.5% confidence intervals and Bonferroni-adjusted p-values (unadjusted p-values multiplied by 2). To better quantify the causal effect of antibiotics, standardised risk differences with 97.5% bootstrap confidence intervals will also be presented.

Priority of covariates

Covariates to include in the model are listed and prioritised in Table 2. Confounders are ranked higher based on importance, i.e. variables which arguably have effect on both outcome (NEC) as well as exposure (decision to start antibiotic treatment, which relates to infection risk/concern). Assessment of covariate importance is based on subject matter knowledge and scientific literature (references in Table 2). For several variables, it is unclear whether there is a relevant effect on NEC and a conservative approach is employed to include potential confounders in the model[15]. Similar considerations apply for assessment of variables with relevant effect on decision to treat with early antibiotics. These variables will be included in the regression for propensity score calculation and subsequent matching. For highly similar variables, the lower priority or quality variables may be omitted if necessary (e.g multi-collinearity issues). Variables with very low quality (e.g. too many missing values) will be omitted. For categorical variables, groups with very few observations will be removed (e.g. separation issues). Estimated effects of each variable included in the model included will be reported. Based on the recommendation to have at least ten events per variable[16], with

the event estimate approximately 1500 cases, this will provide 150 degrees of freedom in the model. Based on the proposed covariates listed in Table 2, the required degrees of freedom for analysis is 108. If the actual number of cases in the obtained dataset is much lower than expected, thus providing insufficient degrees of freedom, covariates may be excluded in reverse order of priority. See detailed specifications of listed covariates/items in Appendix 1.

Table 2. Priority of covariates to include in model based on DAG and availability from NNRD.

		Influence on NEC[17–19]	**Influence on AB-start (decision to treat based on sepsis risk)**[20,21]	**Potential repetition/ redundancy**	**Relation to node in DAG**	**Structure (continuous or number of categories)**
1	**NICU/site**	YES	YES		Site	Random
2	**GA**	YES	YES		GA	Continuous
3	**BW**	YES	YES		BW	Continuous
4	**SEX**	YES	No?		Sex	Dichotomous
5	**APGAR5**	Yes	Yes		Clinical signs	11 categories (0-10)
6	**Delivery mode + expanded (6 categories)**	Yes	YES		Delivery mode and type	6 (see table 1)
7	**Maternal antenatal steroids**	Yes	Yes? (indicator of fetal status/delivery conditions)		Antenatal steroids	None / Incomplete / complete
8	**Feeding first day**	Yes	No		Feeding	1: Enteral feeding on day 1-2, human milk only 2: Enteral feeding on day 1-2, formula only 3: Enteral feeding on day 1-2, mix 3: No enteral feeding on day 1-2
9	IUGR	Yes	Yes		IUGR	Dichotomous (less than -2SDS)
10	APGAR1	Yes	Yes?			11 categories (0-10)
11	APGAR10	Yes	Yes		Clinical signs	11 categories (0-10)
12	EOS	Yes?	No		Sepsis	Dichotomous
13	Birth year (epoch)	Yes	Yes		(similar to site/standards)	4-5
14	Transfer on first day	Yes	Yes		Site/outborn	Dichotomous
15	Level of initial unit	Yes	Yes		Site	Dichotomous
16	Maternal Preeclampsia requiring preterm birth	Yes?	Yes		Preeclampsia	Dichotomous
17	Prolonged ROM	Yes?	YES		Maternal infection	Dichotomous
18	Maternal suspected chorioamnionitis	Yes?	YES	Defined by antibiotics and pyrexia	Maternal infection	Dichotomous
19	Intrapartum antibiotics	Yes?	YES (in relation to chorioamnionitis)		Maternal antibiotics	Dichotomous
20	Maternal pyrexia	Yes?	YES (untreated chorioamnionitis)		Maternal infection	Dichotomous
21	Maternal GBS	Yes?	YES		Maternal infection	Dichotomous
22	Umbilical cord pH	Yes	Yes		Clinical signs	Dichotomous: <7.00 yes or no
23	Umbilical cord lactate	Yes	Yes	Resembles pH	Clinical signs	Cont/ Di/tri?
24	Base excess 12h worst	Yes	Yes		Clinical signs	Dichotomous: < -5 yes/no
25	Umbilical cord base excess	Yes	Yes	Resembles BE 12h worst	Clinical signs	Dichotomous: < -5 yes/no
26	Blood transfusion day 1-2	Yes	Yes?		Anemia	Dichotomous
27	Chest compressions	Yes	Yes?		Clinical signs	Dichotomous
28	Resuscitation drugs at delivery	Yes	Yes?		Clinical signs	Dichotomous
29	Ventilation at delivery	Yes?	Yes? (clinical status at birth)		Assisted ventilation	Dichotomous
30	Spontaneous respiration time	Yes?	Yes?		Clinical signs	3 categories: <1 min, 1-5 min, >5 min
31	Admission temp	Yes	Yes?		Clinical signs	3 categories:

						<36.5, 36.5-37.5, >37.5
32	Admission oxygen SAT	Yes	Yes		Clinical signs	3 categories: >94, 90-94, <90
33	Inotropes on first day	Yes	Yes?		Clinical signs	Dichotomous
34	Admission mean BP	Yes?	Yes/no?	Resembles inotropes	Clinical signs	Dichotomous: below GA yes/no
35	Ethnicity	Yes	Yes? (risk of inf)		Ethnicity	4 categories as suggested in appendix
36	Maternal deprivation score	Yes?	Yes? (risk of inf)		SES	Deprivation centiles?
37	Intubation first day	?	Yes?		Assisted ventilation	Dichotomous
38	Intubation at delivery	?	Yes?	Resembles intubation d1	Assisted ventilation	Dichotomous
39	Surfactant first day	Yes?	Yes?		Surfactant therapy	Dichotomous
40	Surfactant at delivery	?	Yes?	Resembles intubation d1	Surfactant therapy	Dichotomous
41	Time of cord clamp	Yes/No??	Yes? (clinical status at birth)		Clinical signs	Dichotomous: >60 seconds yes/no
42	Probiotics	Yes	No		Probiotic initiation	Dichotomous
43	PDA identified day 1-2	Yes	No		PDA	Dichotomous
44	PDA treatment day 1-2	Yes	No		PDA	Dichotomous
45	Multiplicity	?	No?		Multiplicity	Dichotomous
46	Smoking	Yes?	No?		Smoking	Dichotomous
47	Parity	?	No?		Parity	Dichotomous
48	Umbilical catheters	Yes?	No		Umbilical catheters	Dichotomous
49	Parenteral nutrition d1-2	?	?			Dichotomous
50	Admission heart rate	?	?		Clinical signs	3 categories: >200, 100-200, <100
51	Maternal antenatal magnesium sulphate	No?	Yes/no ?	Resembles preeclampsia	Preeclampsia	Dichotomous
52	Maternal gestational hypertension	No?	No			Dichotomous
53	Maternal diabetes	No?	No		GDM	Dichotomous

Sensitivity analyses

The following sensitivity analyses will be performed:

Early antibiotic exposure only with ampicillin or penicillin plus gentamicin, early antibiotic exposure defined by other timings after birth (later initiation and lasting until 4-6 days after birth) and alternative methods for diagnosing NEC (as standards for NEC diagnosis are unclear). For the latter analyses, we will define and re-analyse NEC diagnosis as 'pragmatic NEC' (5 days of nil by mouth and antibiotics and a diagnostic code of NEC) and NEC including focal intestinal perforation diagnosis (FIP). This condition is sometimes difficult to separate from NEC. We will also record infants with laparotomy- confirmed FIP (intestinal perforation, classified as non-NEC) in addition to the primary NEC (Battersby et al). definition. The statistical analyses will be repeated using propensity score matching (with

propensity scores based on exposure regression), as an alternative approach to logistic regression.

Secondary analyses

We intend to use the same logistic regression models for secondary outcomes, as those specified for the primary outcome. The most important confounders (or proxies) for the secondary outcomes are included in this model. Detailed model specification for each specific secondary outcome as done for the primary outcome is beyond the aim and scope of this study (focusing on NEC). With propensity score matching, direct comparison between antibiotic exposure versus controls can in principle be performed for any outcome, assuming correct model specification for the propensity score.

Exploratory analyses

Additional non-defined exploratory analyses based on findings from the dataset may be performed.

Missing data

We assume that missing data occur randomly between groups and will be imputed ten-fold using multiple imputation by chained equations. Results will be pooled according to Rubin's rule.

Multiple testing

Adjusted P values will be reported with Bonferroni correction of the two primary analyses (along with corresponding 97.5% confidence intervals) and Benjamini-Hochberg adjusted P values from the secondary analyses. Post hoc exploratory analyses will be reported without adjustment of P values and should be interpreted with corresponding caution.

ETHICS AND DISSEMINATION

The study will be registered with International Standard Randomised Controlled Trials Number (ISRCTN) before opening and is sponsored by Newcastle Hospitals NHS Foundation Trust and the protocol with statistical analysis plan will be uploaded to the Open Science Framework website osf.io prior to data analysis initiation. We will apply for HRA/REC approvals. The study is observational and uses de-identified data that is already collected. Dissemination will be by presentation and publication in peer reviewed journals.

PATIENT PUBLIC INVOLVEMENT AND IMPORTANCE TO THE NHS

We have worked closely with parents on all our studies. The NEC UK parent group and other parent groups and representatives continue to assert that better understanding of NEC is a key priority. The NHS, parents and babies experience significant burden from NEC in terms of adverse outcome, prolonged hospitalisation, developmental impact and NHS costs. There is significant concern related to use of antibiotics in the neonatal population and it is important that studies help optimal use of early antibiotics.

DISCUSSION

This study aims to add relevant scientific information to an important clinical decision made for every preterm infant admitted to a neonatal unit: the use and duration of antibiotics in the absence of clear signs of bacteraemia or early onset sepsis. Cases of culture-proven early onset sepsis (EOS) are relatively few, with rates being one to seven per 1000 live births in high income countries[22]. There are potentially large numbers of infants where a clinical choice is available to withhold early antibiotic treatment. Data are currently conflicting as to the overall impact on NEC of receiving (or withholding) antibiotics in the first days of life. Early bacterial nature and load in the preterm gut has been linked to NEC development[23, 24]. Use of intravenous antibiotics shortly after birth may slow colonisation, allowing the gut immune system a short period of adaption that reduces the risk of TLR4 mediated NEC[25]. The integrity of the mucosal barrier has been shown to improve significantly in the first days

after preterm birth in humans[26]. Thus, potentially only short duration of very early antibiotic treatment may be relevant for such effect, in contrast to prolonged treatment which have been shown to cause persistent gut dysbiosis[27] that may instead increase NEC risk[7,8]. Data from a piglet model of NEC suggests that antibiotic use is mechanistically linked to preterm NEC development[28] and preterm immune development[29]. However no difference was seen in total bacterial load of stool in preterm infants who did and did not go on to develop NEC[30]. Given the conflicting data Clinicians need better information to help guide early antibiotic treatment in relation to NEC, especially important as NEC rates in premature infants may actually be increasing[31]. The proposed study using NNRD benefits from access to large numbers of infants with recorded relevant risk factors and outcomes. Large datasets offer the advantage of including many NEC cases, and we anticipate around 1500 informative cases of NEC. These data are increasingly well validated by individual units at the point of data entry, but are potentially less well validated than infants with trial data collected within specific trials.

We have in this study given careful thought to handling confounding factors. Analysis of the current understanding of NEC and the use of directed acyclic graph to guide analysis have been undertaken to attempt to control for what are highly complex clinical factors[17-19]. As demonstrated in the DAG many factors, including those on a causal pathway to NEC, impact the decision to administer early antibiotics[20-21]. The aim to analyse this data using both propensity scoring and logistic regression is a major strength for this study and for future analyses using large databases to address complex questions. Propensity scoring has recently been used to address feeding during hypothermia[32] and the impact of early parenteral nutrition on preterm outcomes[33] using the NNRD, but without alternate statistical approach. Whilst both propensity scoring and regression analysis have strengths and weaknesses to the best of our knowledge direct comparison of these methodologies has not

been undertaken within large neonatal datasets, and is important methodologically for future neonatal studies. The data generated by this study will thus inform important aspects of wider neonatal care and in relation to early neonatal use of antibiotics and later occurrence of NEC.

AUTHOR CONTRIBUTIONS

NE had the original idea for the study. RS undertook the DAG. RS,JF,JB, PTS planned statistical analysis. RS,NE,JF,JB,PTS,CG, GG and SU all contributed to overall study design, protocol development and the writing and review of this paper. JB submitted for registration and approvals.

FUNDING

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COMPETING INTERESTS

No author has relevant competing interests to declare.

DATA STATEMENT

Data may be available on request to the corresponding author.

LEGENDS

Figure 1. Directed Acyclic Graph (DAG) diagram of causal assumptions related to the hypothesis based on subject-matter knowledge, used for confounder selection. Model code text for figure and interactive diagram analysis on dagitty.net is available in Appendix 2. Node with arrowhead: exposure; Node with I: outcome; Black nodes: ancestor of outcome; Dark grey nodes: ancestor of exposure and outcome; White nodes: adjusted variables

(primary analysis); Thick arrow: causal path; Thin arrows: non-biasing paths. AB: early antibiotics; BW: birth weight; GA: gestational age; GDM: gestational diabetes mellitus; IUGR: intrauterine growth restriction; NEC: necrotising enterocolitis; PDA: patent ductus arteriosus; SES: socioeconomic status

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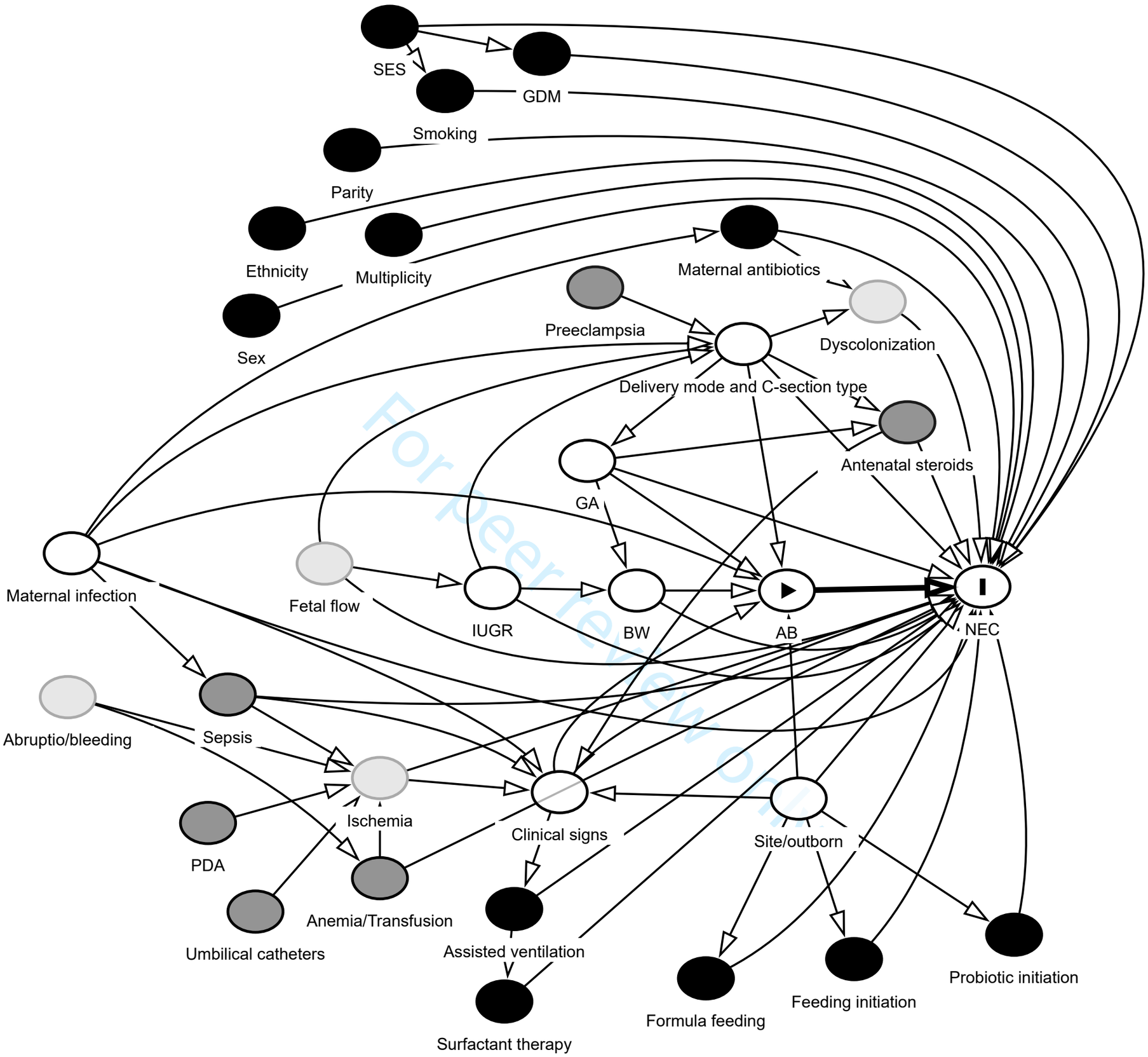

Clevermed code	ICD-10 code	Diagnosis
10741	Q39.0	Oesophageal atresia without distal fistula
16195	Q39.0	Atresia of oesophagus without fistula
10740	Q39.1	Oesophageal atresia with distal tracheo-oesophageal fistula
16196	Q39.1	Atresia of oesophagus with tracheo-oesophageal fistula (TOF)
16197	Q39.2	Congenital tracheo-oesophageal fistula without atresia (TOF)
10273	Q39.3	Congenital stenosis of the oesophagus
16198	Q39.3	Congenital stenosis and stricture of oesophagus
16199	Q39.4	Oesophageal web
10358	Q41.0	Duodenal atresia / stenosis / web (specify)
16212	Q41.0	Congenital absence, atresia and stenosis of duodenum
16213	Q41.0	DA Duodenal atresia / stenosis
10605	Q41.1	Jejunal atresia / stenosis (specify)
16214	Q41.1	JA Jejunal atresia / stenosis
10541	Q41.2	Ileal atresia / stenosis (specify)
16215	Q41.2	Congenital absence, atresia and stenosis of ileum
16216	Q41.2	IA Ileal atresia / stenosis
16217	Q41.X	Congenital absence, atresia and stenosis of small intestine
16218	Q42.0	Congenital absence, atresia and stenosis of rectum with fistula
10496	Q42.00	High anorectal anomaly with rectourethral fistula
10497	Q42.01	High anorectal anomaly with rectovesical fistula
10498	Q42.02	High anorectal anomaly with rectovulval fistula
10495	Q42.03	High anorectal anomaly with rectocutaneous fistula
10494	Q42.04	High anorectal anomaly with rectocloacal fistula
10493	Q42.08	High anorectal anomaly with fistula (specify)
10499	Q42.1	High anorectal anomaly without fistula
16219	Q42.1	Congenital absence, atresia and stenosis of rectum without fistula
16220	Q42.2	Congenital absence, atresia and stenosis of anus with fistula
10636	Q42.20	Low anorectal anomaly with anocutaneous fistula
10637	Q42.21	Low anorectal anomaly with anovestibular fistula
10638	Q42.28	Low anorectal anomaly with fistula (other specify)
10639	Q42.3	Low anorectal anomaly without fistula
16221	Q42.3	Congenital absence, atresia and stenosis of anus without fistula
10240	Q42.31	Congenital anal stenosis
16222	Q42.8	Congenital absence, atresia and stenosis of anus of other parts of large intestine
16223	Q429	Congenital absence, atresia and stenosis of anus of large intestine, part unspecified
16224	Q42X	Congenital absence, atresia and stenosis of large intestine
16235	Q43.7	Persistent cloaca

Supplementary Table 1 Gastrointestinal anomalies

Clevermed code	ICD-10 code	Diagnosis
15890	Q00.0	Anencephaly
15891	Q00.1	Craniorachischisis
15892	Q00.2	Iniencephaly
15893	Q00.X	Anencephaly and similar malformations
15894	Q01.0	Frontal encephalocele
15895	Q01.1	Nasofrontal encephalocele
15896	Q01.2	Occipital encephalocele
15897	Q01.8	Encephalocele of other sites
15898	Q01.9	Encephalocele (unknown or unspecified cause)
15899	Q01.X	Encephalocele
15918	Q04.2	Holoprosencephaly
15926	Q05.0	Cervical spina bifida with hydrocephalus
15927	Q05.1	Thoracic spina bifida with hydrocephalus
15928	Q05.2	Lumbar spina bifida with hydrocephalus
15929	Q05.3	Sacral spina bifida with hydrocephalus
15930	Q05.4	(unknown or unspecified cause) spina bifida with hydrocephalus
15931	Q05.5	Cervical spina bifida without hydrocephalus
15932	Q05.6	Thoracic spina bifida without hydrocephalus
15933	Q05.7	Lumbar spina bifida without hydrocephalus
15934	Q05.8	Sacral spina bifida without hydrocephalus
15935	Q05.9	Spina bifida (unknown or unspecified cause)
10986	Q05.9a	Spina bifida
10704	Q05.9b	Myelomeningocele (specify site)
15936	Q05.X	Spina bifida
16024	Q20.0	Common arterial trunk (Truncus malformation)
10356	Q20.1	Double outlet right ventricle (DORV)
16025	Q20.1	Double outlet right ventricle (DORV)
16026	Q20.2	Double outlet left ventricle (DOLV)
11070	Q20.3	Transposition of the great vessels (TGA)
16027	Q20.3	Transposition great arteries (TGA)
16028	Q20.4	Double inlet ventricle (DILV)
16029	Q20.5	Discordant atrioventricular connection
16030	Q20.6	Is omerism of atrial appendages
16031	Q20.8	Other cong malforms of cardiac chambers and connections
16032	Q20.9	Cong malforms of cardiac chambers and connections unspec
16033	Q20.X	Congenital malformations of cardiac chambers and connections
16035	Q20.91	Atrium single
16036	Q20.92	Ventricle single
10097	Q21.2	Atrio-ventricular septal defect (AVSD)
16039	Q21.2	Atrioventricular septal defect (AVSD)
11043	Q21.3	Tetralogy of Fallot
16040	Q21.3	Tetralogy of Fallot

16045	Q22.0	Pulmonary valve atresia
16046	Q22.1	Congenital pulmonary valve stenosis
16047	Q22.2	Congenital pulmonary valve insufficiency
16048	Q22.3	Other congenital malformations of pulmonary valve
16049	Q22.4	Congenital tricuspid atresia / stenosis
16050	Q22.5	Ebstein's anomaly
16051	Q22.6	Hypoplastic right heart syndrome
16052	Q22.8	Other congenital malformations of tricuspid valve
16053	Q22.9	Congenital malformation of tricuspid valve (unknown or unspecified cause)
16054	Q22.X	Congenital malformations of pulmonary and tricuspid valves
16055	Q23.0	Congenital stenosis of aortic valve (AS)
16056	Q23.1	Congenital insufficiency of aortic valve
16057	Q23.2	Congenital mitral stenosis (MS)
16058	Q23.3	Mitral atresia
16059	Q23.4	Hypoplastic left heart syndrome (HLH)
16060	Q23.8	Other congenital malformations of aortic and mitral valves
16061	Q23.9	Congenital malformation of aortic and mitral valves unspec
16062	Q23.X	Congenital malformations of aortic and mitral valves
16079	Q25.1	Coarctation of aorta
10227	Q25.19	Coarctation of the aorta
16080	Q25.2	Hypoplasia of aortic arch
16081	Q25.3	Stenosis of aorta (AS)
16082	Q25.4	Malformation of aorta
16083	Q25.5	Atresia of pulmonary artery
16084	Q25.6	Stenosis of pulmonary artery (PS)
16086	Q25.8	Other congenital malformations of great arteries
16087	Q25.8	Transposition of the great vessels (TGA)
11057	Q26.2	Total anomylous pulmonary venous drainage (TAPVD)
16092	Q26.2	Total anomalous pulmonary venous connection (TAPVD)
16154	Q33.6	Hypoplasia and dysplasia of lung
16241	Q44.2	Atresia of bile ducts
10123	Q60.1	Bilateral renal agenesis
16318	Q60.1B	Renal agenesis, bilateral
16324	Q60.6	Potter's syndrome
16327	Q61.1	Polycystic kidney, infantile type
10100	Q61.1a	Autosomal recessive polycystic kidney - infantile
10367	Q64.1	Ectopia vesicae
16356	Q64.1	Exstrophy of urinary bladder
10854	Q64.2	Posterior urethral valves (PUV)
16357	Q64.2	Congenital posterior urethral valves (PUV)
16360	Q64.5	Congenital absence of bladder and urethra
10008	Q64.5a	Absence of bladder
10236	Q64.5b	Congenital absence of urethra
16475	Q77.1	Thanatophoric short stature

10246	Q79.0	Congenital diaphragmatic hernia
10490	Q79.0	Hernia into the cord
16495	Q79.0	Congenital diaphragmatic hernia
16496	Q79.1A	Aplasia of diaphragm
16497	Q79.1E	Eventration of diaphragm
16498	Q79.2	Exomphalos
10395	Q79.2	Exomphalos
16499	Q79.3	Gastroschisis
16589	Q90.0	Trisomy 21, meiotic nondisjunction
16590	Q90.1	Trisomy 21, mosaicism (mitotic nondisjunction)
16591	Q90.2	Trisomy 21, translocation
16592	Q90.9	Down's syndrome (unknown or unspecified cause)
16593	Q90.X	Down's syndrome
16594	Q91.0	Trisomy 18, meiotic nondisjunction
16595	Q91.1	Trisomy 18, mosaicism (mitotic nondisjunction)
16596	Q91.2	Trisomy 18, translocation
16597	Q91.3	Edwards' syndrome (unknown or unspecified cause)
16598	Q91.4	Trisomy 13, meiotic nondisjunction
16599	Q91.5	Trisomy 13, mosaicism (mitotic nondisjunction)
16600	Q91.6	Trisomy 13, translocation
16601	Q91.7	Patau's syndrome (unknown or unspecified cause)
16602	Q91.X	Edwards' syndrome and Patau's syndrome

Supplementary Table 2 Congenital anomalies

Early antibiotic use and incidence of necrotising enterocolitis in very preterm infants: a UK based observational study using routinely recorded data

Inclusion criteria: Birth weight < 1500g or GA < 32 wk

Born between: 1/1/2012 and 31/12/2020

Request data as one table with individual-level data with each row representing a unique baby. Each item/variable as separate column.

Exposure parameters:

Requested item	Item name in extract	How is it derived	Coding in extract	Notes
Antibiotics any	Antibiotics_Day1	Postnatal Day 1 with any DailyDrugs in 1010155 Benzyl Penicillin 1010158 Augmentin 1010179 Flucloxicillin 500012 Flucloxacillin 500016 Gentamicin 500072 Co-amoxiclav 500086 Co-amoxiclav 500084 Ciprofloxacin 500029 Netilmicin 500002 Amikacin 500211 Tazocin 500023 Metronidazole 500040 Vancomycin 500007 Cefotaxime 500004 Ampicillin 500009 Cefuroxime 500008 Ceftazidime 500175 Ceftriaxone 500032 Piperacillin 500206 Oflacillin 500005 Azlocillin 1010171 Linezolid 1010271 Cefalexin 1010139 Amoxicillin 500070 Amoxicillin 500128 Meropenem 500118 Imepenem 500145 Imipenem THEN (1)	0 = No 1 = Yes 9 = Unknown	Indicates whether baby has received antibiotics on day 1
	Antibiotics_Day6	As above for each postnatal day between Day2-Day6	0 = No 1 = Yes	

			9 = Unknown	
Antibiotics standard empiric		As above, but only ampicillin or penicillin + gentamicin		
	Day of first antibiotics	Postnatal day of first (of any) antibiotic treatment	Numeric (age in days since birth if received antibiotics) 999 = No antibiotics received throughout admission	
	Total antibiotics	Total number of days on (any) antibiotics throughout admission	Numeric 999 = No antibiotics received throughout admission	

Outcome parameters

Requested item	**Item name in extract**	**How is it derived**	**Coding in extract**	**Notes**
Length of stay		Defined as the total number of days a baby received neonatal care (any level of care) from Daily Care General Information - LOCATIONS OF HIGHEST LEVEL OF CARE	Numeric (age in days at discharge)	
Survival	PostnatalDayofDeath	Where the following is true: DayDateAnon where DischargeDestination = 3	Numeric (age in days since birth if baby died) 999 = Survived	
	Death before day of interest	According to above - Before day 3 (exclusion) - Before day 7 (for competing risk analysis) - Before day 14 (for competing risk analysis) - Before day 28	0 = Yes 1 = No 9 = Unknown	
	SurvivaltoDischarge	Where the following is true: DischargeDestination = 3 THEN (0)	0 = Died 1 = Survived to discharge 9 = Unknown	Survival to discharge from neonatal care for the final episode
	FinalDischDestination	N/A	Text	Discharge destination for the final episode
Cause of death	Causeofdeath	ICD-10		
Necrotising enterocolitis (possible more than one episode)	NEC_NNAP	Where the following is true: NECTreatment > 0 AND XRayAppearances in	0 = No NEC 1 = NEC present 9 = Unknown	NNAP definition

		Pneumatosis Pneumoperitoneum AND ClinicalFindings in Increased/bilious aspirate Abdominal distension Bloody stools THEN (1)		
	Severe_NEC_Battersby	Where the following is true: CauseOfDeath = 17 OR PortMortemConfirmation = 1 OR GastrointestinalDiagnoses OR PrincipleProceduresDuringStay OR PrincipalDiagnosisAtDischarge in Laparotomy Laparotomy approach NEC Colectomy and ileostomy NEC AND GastrointestinalDiagnosis or PrincipalDiagnosisAtDischarge in Necrotising enterocolitis Necrotising enterocolitis – confirmed Necrotising enterocolitis – perforated Necrotising enterocolitis – proven (on xray or surgery) OR GastrointestinalDiagnoses OR PrincipalDiagnosisAtDischarge in Necrotising enterocolitis Necrotising enterocolitis – confirmed Necrotising enterocolitis – perforated Necrotising enterocolitis – proven (on xray or surgery) AND DischargeDestination = 3 OR GastrointestinalDiagnoses or PrincipalDiagnosisAtDischarge = Necrotising enterocolitis – perforated OR NECTreatment >= 1 AND GastrointestinalDiagnoses OR PrincipalDiagnosisAtDischarge in Necrotising enterocolitis Necrotising enterocolitis – confirmed Nectotising enterocolitis – perforated Necrotising enterocolitis – proven (on xray or surgery)	0 = No severe NEC 1 = Severe NEC present 9 = Unknown	Battersby Severe NEC definition

		OR LaparotomyPerformed = Yes AND HistologyConfirmationNEC = Yes OR VisualInspectionConfirmationNEC = Yes THEN (1)		
	NEC_Any (pragmatic)	Where the following is true: (TreatmentNEC >= 1 OR Code in 1010683 Necrotising enterocolitis – suspected 10708 Necrotising enterocolitis – Perforated 15809 Necrotizing enterocolitis 15809 Necrotising enterocolitis - Confirmed) AND (5 or more days of nil by mouth where DayEnteralFeeds = 0 OR DayFormulaType = No entry OR VolumeMilk = 0/No entry AND 5 consecutive days of antibiotics over the same five days of receiving nil by mouth where DailyDrugs in 1010155 Benzyl Penicillin 1010158 Augmentin 1010179 Flucloxicillin 500012 Flucloxacillin 500016 Gentamicin 500072 Co-amoxiclav 500086 Co-amoxiclav 500084 Ciprofloxacin 500029 Netilmicin 500002 Amikacin 500211 Tazocin 500023 Metronidazole 500040 Vancomycin 500007 Cefotaxime 500004 Ampicillin 500009 Cefuroxime 500008 Ceftazidime 500175 Ceftriaxone 500032 Piperacillin	0 = No NEC present 1 = NEC present 9 = Unknown	Webbe NEC definition for any NEC treatment

		500206 Oflacillin 500005 Azlocillin 1010171 Linezolid 1010271 Cefalexin 1010139 Amoxicillin 500070 Amoxicillin 500128 Meropenem 500118 Imepenem 500145 Imipenem OR Died within 5 days of ' TreatmentNEC >= 1')		
	NEC onset day	Postnatal age for NEC onset TreatmentNEC >= 1 OR First day where diagnosis 1010683 Necrotising enterocolitis – suspected 10708 Necrotising enterocolitis – Perforated 15809 Necrotizing enterocolitis 15809 Necrotising enterocolitis – Confirmed is recorded		
	NEC onset PMA	Postmenstrual age for NEC onset		
NEC related variables (raw data)	PostmortemConfirmation	If a necrotising enterocolitis diagnosis was made at any point at admission, specify if the post mortem confirmed it.	N No Y Yes 9 Unknown	
	SurgicalProcedure	Surgical procedure on the date and time specified OPCS coded and/or SNOMED CT		
	StomaInSitu	N No Y Yes		
	InvestigateAbdsigns	N No Y Yes		
	XrayAppearance	?? 1 Pneumatosis 2 Air in the liver 3 Pneumoperitoneum 4 Fixed loop 5 Gasless 9 None of the above		
	abdominalxrayfindings	01 Abdominal distension 02 Abdominal tenderness 03 Increased/ bilious aspirates 04 Abdominal discolouration 05 Abdominal mass 06 Bloody stools		

		07 Mucousy stools 09 None of the above		
	TransferredOutManagementNEC	N No Y Yes		
	necLaparotomy	0 Laparotomy not required 1 Laparotomy required but PATIENT too ill to carry it out 2 Laparotomy required and carried out		
	laparotomyConfirm	N No Y Yes		
	necHistologyConfirmed	0 Not confirmed 1 Yes confirmed 9 No histological inspection/Not applicable		
Infant: Sepsis suspected on first day	SuspectedSepsisFirstDays	Where the following is true: DayDateAnon in first day of life AND first full day in unit AND SuspectedSepsis >= 1 (Y)	0 = No 1 = Yes 9 = Unknown	
Early onset blood stream infection		Defined from Infection Cultures (Episodic) recorded in the first 3 days/before day 3 • Pure growth of pathogen from blood OR • Pure growth of pathogen from CSF OR • Either a pure growth of a skin commensal or a mixed growth with ≥3 clinical signs at the time of blood sampling		
Late onset blood stream infection NNAP definition		Defined from Infection Cultures (Episodic) recorded after day 3 • Pure growth of pathogen from blood OR • Pure growth of pathogen from CSF OR • Either a pure growth of a skin commensal or a mixed growth with ≥3 clinical signs at the time of blood sampling	Dichotomous (No infection=0, Infection=1) Dichotomous Unknown = 9	
Late onset infection, non-NNAP		5 consecutive days of antibiotic treatment defined as 5 consecutive days of any of the following (including in combination and changing during the 5 days) after day 3 Daily care medication •1010155 Benzyl Penicillin •1010158 Augmentin •1010179 Flucloxicillin •500012 Flucloxacillin •500016 Gentamicin •500072 Co-amoxiclav •500086 Co-amoxiclav •500084 Ciprofloxacin	Dichotomous (No infection=0, Infection=1) Dichotomous	

		•500029 Netilmicin •500002 Amikacin •500211 Tazocin •500023 Metronidazole •500040 Vancomycin •500007 Cefotaxime •500004 Ampicillin •500009 Cefuroxime •500008 Ceftazidime •500175 Ceftriaxone •500032 Piperacillin •500206 Oflacillin •500005 Azlocillin •1010171 Linezolid •1010271 Cefalexin •1010139 Amoxicillin •500070 Amoxicillin •500128 Meropenem •500118 Imepenem •500145 Imipenem •500069 Ambisome (Liposomal Amphotericin) •500003 Amphotericin •1010195 Amphotericin Liposoma		
Early onset infection (pragmatic)		>5 consecutive days of antibiotic treatment defined as 6 consecutive days of any of the following (including in combination and changing during the 6 days) before day 3 Daily care medication		
Time to reach full feeding		First day of 3 consecutive days where parenteral nutrition or intravenous fluid are not recorded		

Covariates/confounders

BadgerID	AnonPatientID		N/A	Episode-specific identifier for each baby
Gestational age at birth	GestationWeeks		Numeric (10 - 49)	
	GestationDays		Numeric (0-6) 9 = Unknown	
	GA total days	Weeks x 7 + days		

Birth weight	Birthweight		Numeric (g) 99999 = Unknown	Accepted range: 001 – 9998g
Birth head circumference	BirthHeadCircumference	Can perhaps be useful to differentiate between symetrical and asymetrical IUGR		
Sex	Gender		0 = Unknown 1 = Male 2 = Female 9 = Not specified	
Birth weight z-score		Specify Marsal or Fenton or WHO	Numeric 99999 = Unknown	
Ethnicity	Race	Combine parental ethnicities (see row 36,39) Parents Demographics ETHNIC CATEGORY (MOTHER) (categorical) Coded as: WHITE (A - British, B - Irish, C - Any other white background); MIXED (D - White and Black Caribbean. E - White and Black African, F - White and Asian, G - Any other mixed background); ASIAN OR ASIAN BRITISH (H - Indian, J - Pakistani, K - Bangladeshi, L - Any other Asian Background); BLACK OR BLACK BRITISH (M - Caribbean, N - African, P - Any other Black background); OTHER ETHNIC GROUPS (R - Chinese, S - Any other ethnic group); UNKNOWN (Z, DTA - Not stated, 99 - Not known) This data item is based on self-reported ethnicity as recorded in maternity notes	?e.g. Categorised into four groups (White=1; Asian & Mixed=2; Black & Mixed=3; Other and not given=4)	
Smoking during pregnancy	Smoking	Pregnancy Details MOTHER CURRENT SMOKER AT BOOKING INDICATOR (categorical, codes 1-6)	0 = No 1 = Yes 9 = Unknown	
Multiplicity	FetusNumber	N/A	Numeric 99 = Unknown	
Birth year	BirthYear	N/A	Numeric 9999 = Unknown	
Birth year (mother)	BirthYearMother	N/A	Numeric 9999 = Unknown	
Parity of mother	Primiparity	Pregnancy Details PREGNANCY TOTAL PREVIOUS PREGNANCIES Dichotomous: code 00=Y; code 01- 29=N	Primiparous: Dichotomous (Not first pregnancy=0; First pregnancy=1)	
Maternal deprivation score	PostCodeMother	LSOA centiles		
	MumEducation			
	MumOccupation			
Maternal Diabetes (Y/N)	MaternalDiabetes	Where the following is true: ProblemsMedicalMother = 15 THEN (1) ProblemsMedicalMother = 00 THEN (0)	0 = No maternal diabetes 1 = Maternal Diabetes Present 99 = Unknown	Blank entries coded in as 99

Maternal gestational diabetes (Y/N)	MaternalGestDiabetes	Where the following is true: ProblemsDuringPregnancy = 33 THEN (1) ProblemsDuringPregnancy = 00 THEN (0)	0 = No gestational diabetes 1 = Gestational diabetes present 99 = Unknown	Blank entries coded in as 99
Maternal pre-eclampsia requiring pre-term birth (Y/N) Maternal pre-eclampsia	PreEclampsia	Where the following is true: ProblemsDuringPregnancy = 31 THEN (1)	0 = No pre-eclampsia 1 = Pre-eclampsia present 99 = Unknown	Blank entries coded in as 99
Maternal gestational hypertension (Y/N)	MaternalGestHypTension	Where the following is true: ProblemsDuringPregnancy = 30 THEN (1)	0 = No gestational hypertension 1 = Gestational hypertension present 99 = Unknown	Blank entries coded in as 99
Maternal prolonged rupture of membranes	ROMTimeAnon	Derived (Minutes)	Numeric	Number of minutes from birth to event
	Prolonged_ROM	Where the following is true: ProblemsDuringPregnancy = 20 THEN (1)	0 = No prolonged rupture 1 = Prolonged rupture present	
Maternal suspected chorioamnionitis (Y/N)	Chorioamnionitis	Where the following is true: MaternalPyrexiaInLabour38c = 1 OR IntrapartumAntibioticsGiven = 1 THEN (1)	0 = No 1 = Yes 9 = Unknown	Blank entries coded in as 9
Intrapartum Antibiotics	IntrapartumAntibioticsGiven	IntrapartumAntibioticsGiven = 1 THEN (1)	0 = No 1 = Yes 9 = Unknown	
Maternal pyrexia	MaternalPyrexiaInLabour	MaternalPyrexiaInLabour38c = 1 THEN (1)	0 = No 1 = Yes 9 = Unknown	
Maternal GBS	MaternalGBS	ProblemsInfctPregnancyMother = Group B streptococcus THEN (1)	0 = No 1 = Yes 9 = Unknown	
Maternal receipt of antenatal steroids (Y/N)	MaternalAntenatalSteroids	Where the following is true: SteroidsAntenatalGiven = 1 AND SteroidsAntenatalCourses = 1 THEN (1) SteroidsAntenatalGiven = 1 AND SteroidsAntenatalCourses = 2 THEN (2) SteroidsAntenatalGiven = 0 AND SteroidsAntenatalCourses = 0 THEN (0)	0 = None given 1 = Complete 2 = Incomplete 9 = Unknown	
Maternal receipt of antenatal magnesium sulphate (Y/N)	MagnesiumSulphate	N/A	0 = No 1 = Yes 9 = Unknown	

Delivery mode	ModeOfDelivery	Emergency caesarean = 1 Elective caesarean = 2 Vaginal spontanous = 3	1 = emergency 2 = elective 3 = vaginal 9 = unknown	
Induced delivery	Onsetoflabour	Spontanous = 1 Induced (medical and/or surgical) = 2 None (i.e. caesarean) = 3	1 = spontanous 2 = Induced 3 = None 9 = unknown	
Labour before caesarean	ModeofDelivery Caesarean	Yes No	0 = no labour 1 = labour	
Delivery categories	Delivery categories	Categorize above: Vaginal AND Spontanous = 1 Vaginal AND Induced = 2 Emergency caesarean AND labour = 3 Emergency caesarean AND nolabour = 4 Elective caesarean AND labour = 5 Elective caesarean AND nolabour = 6	1 = VagSpon 2 = VagInduc 3 = EmergCaesLab 4 = EmergCaesNolab 5 = ElectCaesLab 6 = ElectCaesNolab 9 = unknown	
Infant Apgar score at 1 minutes	apgar_1min	N/A	0-10 Apgar score 99 = Unknown	
Infant Apgar score at 5 minutes	apgar_5min	N/A	0-10 Apgar score 99 = Unknown	
Infant Apgar score at 10 minutes	apgar_10min	N/A	0-10 Apgar score 99 = Unknown	
Spontaneous respiration time of onset	SpontaneousRespirationTime	1 <1 mins 2 1-1.5 mins 3 1.6-2 mins 4 2.1-3 mins 5 3.1-4 mins 6 4.1-5 mins 7 > 5mins		
Infant: chest compressions administered (Y/N)	CardiacMassage	Where the following is true: MethodsOfResuscitation = 16 THEN (1)	0 = No cardiac massage 1 = Cardiac massage 99 = Unknown	
Infant: Intubation at delivery (Y/N)	IntubationDelivery	Where the following is true: MethodsOfResuscitation = 15 THEN (1)	0 = No intubation 1 = Intubation 99 = Unknown	
Infant: Ventilation at delivery (Y/N)	VentilationDelivery	Where the following is true: MethodsOfResuscitation = 14 THEN (1)	0 = No IPPV 1 = IPPV 99 = Unknown	
Infant: Emergency resuscitation drugs administered (Y/N)	ResusDrugsAdmin	Where the following is true: MethodsOfResuscitation = 17 OR MethodsOfResuscitation = 88 THEN (1)	0 = No resuscitation drugs administered	

			1 = Resuscitation drugs administered 99 = Unknown	
Infant: Surfactant administered (Y/N)	SurfactantGivenResuscitation	N/A	0 = No 1 = Yes 9 = Unknown	
Infant: Umbilical cord pH	CordPhArterial	N/A	6.00-8.00 9.99 = Unknown	
	CordVenousPH	N/A	6.00-8.00 9.99 = Unknown	
Umbilical cord lactate	CordlLactate			
Umbilical cord base excess	CordBE	Labour and Delivery Details UMBILICAL CORD BLOOD BASE EXCESS CONCENTRATION (ARTERIAL) Continuous OR if not available use Labour and Delivery Details UMBILICAL CORD BLOOD BASE EXCESS CONCENTRATION (VENOUS)	CordBaseExcess: Continuous (to 1 decimal place) CordBaseExcessMs: Binary missing indicator created (Not missing=0; Missing=1)	
Base excess 12h worst	WorstBaseWithin12			
Blood transfusion day 1-2	BloodProductsTrans	On day 1 and day 2 Daily care blood transfusion BLOOD TRANSFUSION PRODUCT TYPE	1 = yes 0 = no	
Umbilical catheters	LinesIn	Only on day 1-2 (admission) 1 Peripheral arterial line 2 Umbilical arterial line if THEN YES 3 Umbilical venous line if THEN YES 4 Percutaneous central venous line (long line) 5 Surgically inserted central venous line 6 Peripheral venous line 9 Not Applicable/ No Lines in Situ	1 = yes 0 = no 9 = unknown	
Birth place	PlaceofBirthNHSCode		Organization code	
Time to admission		Admission Details CRITICAL CARE START YEAR AND MONTH and NUMBER OF MINUTES (BIRTH TO EVENT)		
Assisted Ventilation		On day 1-2		
Surfactant therapy		On day 1-2		
Feeding advancement (early feeding)		198:9 E.g. any of the following items entered in the Daily Care Fluids and Feeding during the first 3 days • Any entry (1-6) under ENTERAL FEED TYPE GIVEN	Dichotomous (No enteral feeds=0; provided enteral feeds=1)	

		• OR any entry (0-88) under FORMULA MILK OR MILK FORTIFIER TYPE • OR any value >0 for TOTAL VOLUME OF MILK RECEIVED • OR any entry (1-8) under ENTERAL FEEDING METHOD NO ENTERAL FEEDING GROUP DEFINED AS All other babies not fulfilling above criteria		
Feeding		On day 1-2 1 Suckling at the breast 2 Mother's fresh expressed breast milk 3 Mother's frozen expressed breast milk 4 Donor expressed breast milk 5 Breast milk fortifier 6 Formula 9 Not applicable (Nil by mouth)	1 = Formula 2 = Human milk 3 = Mix ? 0 = nil 9 = unknown	
Probiotics		On day 1-2	1 = yes 0 = no	
Fluids and feeding: Parenteral nutrition today (partial or total)	ParenteralNutrition	PN on day1-2	1= yes 0 = no	
Unit of first admission	ProviderNHSCode	N/A	xxxxx - NHS organisational code ZZ210 - non-NHS England and Wales organisation (private/N.Ireland/Scotland) ZZ203 – Not known ZZ999 - Missing	
Infant: Admission temperature	FirstAdmitTemperature	N/A	24-45 77.7 = Not Recordable	Measurement at first admission
Infant: Admission mean blood pressure	FirstAdmissionBP	N/A	10-150 999 = Unknown	Measurement at first admission
Infant: Admission blood glucose	FirstAdmissionBloodGlucose	N/A	0.0-50.0 99.9 = Unknown	Measurement at first admission
Infant: Admission heart rate	FirstAdmissionHR	N/A	50-350 999 = Unknown	Measurement at first admission
Infant: Admission oxygen saturation	FirstAdmissionOxygenSaturation	N/A	10-100 999 = Unknown	Measurement at first admission

Infant: Surfactant administered on first day (Y/N)	SurfactantGivenFirstDays	Where the following is true: DayDateAnon in first day of life AND first full day in unit AND SurfactantGiven = 1 THEN (Y)	0 = No 1 = Yes 9 = Unknown	
Infant: Mechanical ventilation on first day (Y/N)	RespiratorySupportFirstDays	Where the following is true: DayDateAnon in first day of life AND first full day in unit AND RespiratorySupport = 1 OR AddedO2 = 11 OR VentilationMode >= 1 THEN (1)	0 = No mechanical ventilation on first day 1 = Mechanical ventilation on first day 9 = Unknown	Blank entries coded as 9
Infant: Inotropes administered on first day (Y/N)	InotropesFirstDays	Where the following is true: DayDateAnon in first day of life AND first full day in unit AND DailyDrugs in 500098 Dopamine 500096 Dobutamine 500056 Adrenaline 500210 Noradrenaline 500116 Hydrocortisone 1010173 Milrinone OR InotropesGiven = 1 THEN (Y)	0 = No 1 = Yes, Inotropes given today 9 = Unknown	Blank entries coded in as 9
Hemodynamically significant PDA	PDA	Treated on day 1-2 Yes/No		
	Cardiovascular: Treatment for patent ductus arteriosus (PDA)	1 Indometacin/Indomethacin 2 Ibuprofen 3 Surgery 9 Not applicable		
Neurology: Central tone	Centraltone	At admission (On day 1-2) 1 Normal 2 Increased 3 Decreased	0 = normal 1 = abnormal incl floppy	
Admission: Time of cord clamping	CordClamp	Cord clamped immediately after birth	0 = No 1= Yes 9 = Unknown	
Infant: Transfer on first day (Y/N)	TransferOnFirstDay	Where the following is true: AdmitTimeAnon <= 1440 AND DischTimeAnon <= 1440 AND DischargeDestination does not equal 3 AND ProviderCode is different from POBCode AND EpisodeNumberBaby = 2 THEN (1)	0 = No 1 = Yes 9 = Unknown	

		Same as: Admission Details SITE CODE (OF ADMITTING NEONATAL UNIT) or ORGANISATION CODE (OF ADMITTING NEONATAL UNIT) Different from Baby Demographics SITE CODE (OF ACTUAL PLACE OF DELIVERY) or ORGANISATION CODE (OF ACTUAL PLACE OF DELIVERY) And Baby Demographics EPISODE NUMBER		
	TransferDestination	Derived from ProviderNHSCode	xxxxx - NHS organisational code ZZ210 - non-NHS England and Wales organisation (private/N.Ireland/Scotland) ZZ203 – Not known ZZ999 - Missing	
Level of initial neonatal unit	POBLevel	Derived from POBNHSCode	0 = Non-NNU 1 = SCU 2 = LNU 3 = NICU	
Neonatal network	POBNetwork	Derived from POBNHSCode	Text	

Additional outcomes

Brain injury on imaging	BrainInjuryImaging	Where the following is true: LeftIVH OR RightIVH >= 3 OR PVL = Y THEN (1)	0 = No brain injury on imaging 1 = Brain injury on imaging 9 = Unknown	Blank entries coded as 9
Treated ROP	Treated_ROP	Where the following is true: ROPSurgery = 1 OR RightEyeSurgery = 1,2,3,4 OR LeftEyeSurgery = 1,2,3,4	0 = No treatment 1 = Treatment given	
Maximum stage of ROP	Max_ROP	N/A	0 – No ROP 1 – Stage 1 ROP 2 - Stage 2 ROP 3 – Stage 3 ROP 4 – Stage 4 ROP 5 – Stage 5 ROP A – Aggressive posterior ROP	The maximum stage between right and left eye

Bronchopulmonary dysplasia	BPD	Where the following is true: CGA = 36 calculated from GestationWeeks and GestationDays AND RespiratorySupport = 1 OR VentilationMode >= 1 OR Added02 > 0 THEN (1)	0 = No respiratory support given at 36 weeks 1 = Respiratory support at 36 weeks 9 = Unknown	Blank entries coded as 9
Need for surgical procedures (possible more than one)	SurgicalProcedures	Where the following is true: PrincipleProceduresDuringStay = 100033 Surgery for meconium ileus (von) 100076 Skin or soft tissue surgery requiring general or spinal anaesthesia (Description Required) 11222 Closure of small intestine/ileal perforation 11501 Laparoscopy 11904 Colostomy 11905 Ileostomy 1010826 Major surgery OR MajorSurgeryToday = Y THEN (1)	0 = No 1 = Yes 9 = Unknown	Blank entries coded as 9
Seizures	Seizures	Where the following is true: PrincipalDiagnosisAtDischarge = 10957 Seizures 15192 Seizure disorder 15194 Seizure disorder (cause unknown) 15195 Status epilepticus 15848 Seizures OR Convulsions = 1 THEN (1)	0 = None 1 = Seizures at discharge 9 = Unknown	Blank entries coded as 9
Weight at 36 weeks corrected gestational age (CGA)	CGA_weight	Where the following is true: DayWorkingWeight at 36 weeks (+/- 3 days) CGA	Numeric (g) 99999 = Unknown	Accepted range: 001 – 9998g
Exact CGA on day of measurement	CGA_exact	Calculated from DayDateAnon and GestationWeeks and GestationDays	Numeric	
Weight at discharge	DischWeightFinalEps	DayWorkingWeight on the last day of the last episode	Numeric (g) 99999 = Unknown	Accepted range: 001 – 9998g
Weight SDS at discharge		Defined as the following data item on the final day of neonatal care: • Daily Care General Information PERSON WEIGHT IN GRAMS If final day is not entered, the penultimate day is used	Continuous	
Head circumference at discharge	DischHeadCircumFinalEps	DayHeadCirc on the last day of the last episode	Numeric (cm) 99.9 = Unknown	
CGA day of discharge	CGA_DischFinalEps	Calculated from DayDateAnon and GestationWeeks of the last episode	Numeric	

Blindness	Vision_impairment	Where the following is true: Vision_visual_problems = 1 OR vision_defect_not_correctable = 1 OR Vision_blind = 1 THEN (1)	0 = No 1 = Yes 9 = Unknown	
Deafness	auditory_hearing_impairment	N/A	0 = No 1 = Yes 9 = Unknown	
Ability to walk	neuromotor_unable_walk_without_a	N/A	0 = No 1 = Yes 9 = Unknown	
Exclusions for congenital gastrointestinal malformations	Exclusions_Malformations	Where the following is true: Diagnosis table code in eTable1 THEN (1)	0 = No, none of the listed malformations present 1 = Yes	
Exclusions for life-limiting conditions or conditions requiring surgery	Exclusions_LifeLimitingOrSurgery	Where the following is true: Diagnosis table code in eTable2 THEN (1)	0 = No, none of the conditions present 1 = Yes	
Clinical diagnoses at discharge	DiagnosesAtDischarge	ICD-10 and/or SNOMED CT Can be derived from daily records. [Secondary/exploratory outcomes]	For each selected outcome 0=No 1=Yes	

Appendix 2. DAG code for dagitty.net (for direct insertion on website browser platform)

```
dag {
bb="0,0,1,1"
"Abruptio/bleeding" [latent,pos="0.146,0.569"]
"Anemia/Transfusion" [pos="0.304,0.710"]
"Antenatal steroids" [pos="0.571,0.355"]
"Assisted ventilation" [pos="0.372,0.734"]
"Clinical signs" [adjusted,pos="0.395,0.643"]
"Delivery mode and C-section type" [adjusted,pos="0.488,0.294"]
"Feeding initiation" [pos="0.544,0.773"]
"Fetal flow" [latent,pos="0.276,0.465"]
"Formula feeding" [pos="0.469,0.788"]
"Maternal antibiotics " [pos="0.491,0.203"]
"Maternal infection" [adjusted,pos="0.148,0.457"]
"Probiotic initiation" [pos="0.625,0.754"]
"Site/outborn" [adjusted,pos="0.516,0.648"]
"Surfactant therapy" [pos="0.367,0.806"]
"Umbilical catheters" [pos="0.241,0.736"]
AB [exposure,pos="0.510,0.486"]
BW [adjusted,pos="0.434,0.486"]
Dyscolonization [latent,pos="0.556,0.261"]
Ethnicity [pos="0.252,0.204"]
GA [adjusted,pos="0.409,0.385"]
GDM [pos="0.386,0.068"]
IUGR [adjusted,pos="0.361,0.484"]
Ischemia [latent,pos="0.304,0.632"]
Multiplicity [pos="0.311,0.209"]
NEC [outcome,pos="0.609,0.483"]
PDA [pos="0.217,0.667"]
Parity [pos="0.290,0.143"]
Preeclampsia [pos="0.413,0.250"]
SES [pos="0.309,0.047"]
Sepsis [pos="0.227,0.567"]
Sex [pos="0.239,0.272"]
Smoking [pos="0.337,0.097"]
"Abruptio/bleeding" -> "Anemia/Transfusion" [pos="0.243,0.615"]
"Abruptio/bleeding" -> Ischemia
"Anemia/Transfusion" -> Ischemia
"Anemia/Transfusion" -> NEC
"Antenatal steroids" -> "Clinical signs" [pos="0.489,0.410"]
"Antenatal steroids" -> NEC
"Assisted ventilation" -> "Surfactant therapy"
"Assisted ventilation" -> NEC
"Clinical signs" -> "Assisted ventilation"
"Clinical signs" -> AB [pos="0.386,0.578"]
"Clinical signs" -> NEC [pos="0.423,0.577"]
"Delivery mode and C-section type" -> "Antenatal steroids"
"Delivery mode and C-section type" -> AB
"Delivery mode and C-section type" -> Dyscolonization
"Delivery mode and C-section type" -> GA
"Delivery mode and C-section type" -> NEC
"Feeding initiation" -> NEC [pos="0.601,0.675"]
```

Appendix 2. DAG code for dagitty.net (for direct insertion on website browser platform)

```
"Fetal flow" -> "Delivery mode and C-section type"
[pos="0.261,0.334"]
"Fetal flow" -> IUGR
"Fetal flow" -> NEC [pos="0.385,0.603"]
"Formula feeding" -> NEC [pos="0.555,0.719"]
"Maternal antibiotics " -> Dyscolonization
"Maternal antibiotics " -> NEC [pos="0.642,0.236"]
"Maternal infection" -> "Clinical signs" [pos="0.362,0.575"]
"Maternal infection" -> "Delivery mode and C-section type"
[pos="0.238,0.289"]
"Maternal infection" -> "Maternal antibiotics " [pos="0.229,0.267"]
"Maternal infection" -> AB [pos="0.322,0.356"]
"Maternal infection" -> NEC [pos="0.575,0.705"]
"Maternal infection" -> Sepsis
"Probiotic initiation" -> NEC [pos="0.639,0.652"]
"Site/outborn" -> "Clinical signs"
"Site/outborn" -> "Feeding initiation"
"Site/outborn" -> "Formula feeding"
"Site/outborn" -> "Probiotic initiation"
"Site/outborn" -> AB
"Site/outborn" -> NEC
"Surfactant therapy" -> NEC
"Umbilical catheters" -> Ischemia
AB -> NEC
BW -> AB
BW -> NEC [pos="0.526,0.569"]
Dyscolonization -> NEC [pos="0.604,0.306"]
Ethnicity -> NEC [pos="0.720,0.028"]
GA -> "Antenatal steroids"
GA -> AB
GA -> BW
GA -> NEC
GDM -> NEC [pos="0.806,0.106"]
IUGR -> "Delivery mode and C-section type" [pos="0.319,0.358"]
IUGR -> BW
IUGR -> NEC [pos="0.512,0.614"]
Ischemia -> "Clinical signs"
Ischemia -> NEC
Multiplicity -> NEC [pos="0.697,0.057"]
PDA -> Ischemia
Parity -> NEC [pos="0.756,0.077"]
Preeclampsia -> "Delivery mode and C-section type"
SES -> GDM
SES -> NEC [pos="0.852,0.023"]
SES -> Smoking
Sepsis -> "Clinical signs" [pos="0.346,0.586"]
Sepsis -> Ischemia
Sepsis -> NEC [pos="0.478,0.596"]
Sex -> NEC [pos="0.690,0.026"]
Smoking -> NEC [pos="0.780,0.096"]
}
```

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **p2 L10**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **NA**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **P4**
Objectives	3	State specific objectives, including any prespecified hypotheses **P5 line 6-10**
Methods		
Study design	4	Present key elements of study design early in the paper **P5 Line 11 onwards**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **P5 L15-21**
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up **P5 L22-26**
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed **P8 L1 - 5**
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **P8 L7 on and table 1**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **Table 1**
Bias	9	Describe any efforts to address potential sources of bias **P9 -13**
Study size	10	Explain how the study size was arrived at **P7 L23 – P8 L5**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **P9**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **P9 -13**
		(*b*) Describe any methods used to examine subgroups and interactions **P12 L2 -12**
		(*c*) Explain how missing data were addressed **P14 L14-17**
		(*d*) If applicable, explain how loss to follow-up was addressed **NA**
		(*e*) Describe any sensitivity analyses **P12 L2 – P13 L2**
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **NA**
		(b) Give reasons for non-participation at each stage **NA**
		(c) Consider use of a flow diagram **NA**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **NA**
		(b) Indicate number of participants with missing data for each variable of interest
		(c) Summarise follow-up time (eg, average and total amount) **NA**
Outcome data	15*	Report numbers of outcome events or summary measures over time **NA**
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **NA**

		(*b*) Report category boundaries when continuous variables were categorized **NA**
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period **NA**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **NA**
Discussion		
Key results	18	Summarise key results with reference to study objectives **NA**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential **bias P15 L20 - 25**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **NA**
Generalisability	21	Discuss the generalisability (external validity) of the study results **P15 L10 -15**
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **P16 L11-14**

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.