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Evidence for causal associations between prenatal and postnatal antibiotic exposure and asthma in children, England

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Abstract

Background: Higher risks of asthma have been observed in children with prenatal exposure to antibiotics and during early life compared with those who have not. However, the causality of such associations is unclear.

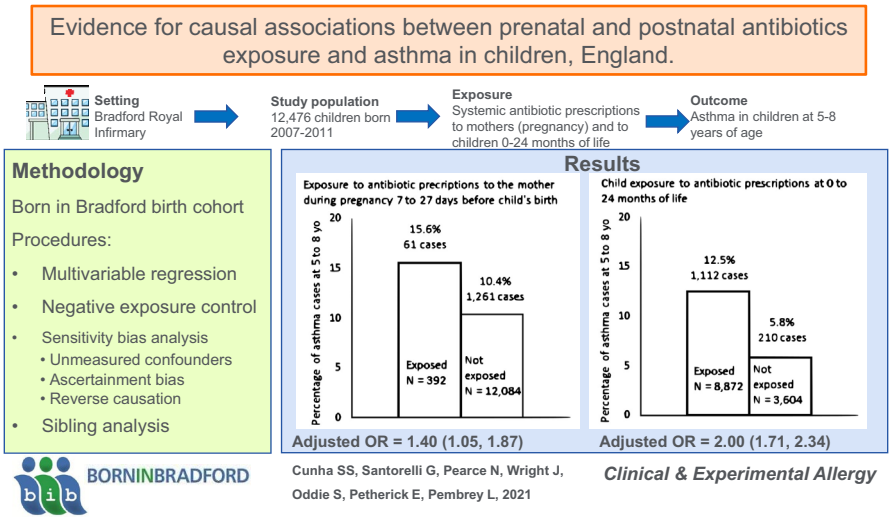
Objective: To assess whether exposure to antibiotics in early life had a causal effect in increasing the risk of asthma in children diagnosed at 5–8 years of life, and the impact in the target population.

Methods: Data were from electronic health records and questionnaires for children and their mothers in the Born in Bradford birth cohort. Exposure variables were prescriptions of systemic antibiotics to the mother during pregnancy (prenatal) and to the children at 0–24 months of life (postnatal). We assessed the association in 12,476 children with several approaches to deal with different sources of bias (triangulation): the interactions with mother's ethnicity, mode of delivery, and between prenatal and postnatal exposures; dose-response; and estimated the population attributable risk.

Results: There was an association between prenatal exposure at 7–27 days before the child's birth and asthma (adjusted OR = 1.40; 1.05, 1.87), but no association with the negative control exposure (before pregnancy) (adjusted OR = 0.99 (0.88, 1.12)). For postnatal exposure, the adjusted OR was 2.00 (1.71, 2.34), and for sibling analysis, it was 1.99 (1.00, 3.93). For postnatal exposure, the risk of asthma increased with the number of prescriptions. The observed effect of both exposures was lower among children with mothers of Pakistani ethnicity, but inconclusive ($p > .25$). The interaction between prenatal and postnatal exposures was also inconclusive ($p = .287$). The population attributable risk of postnatal exposure for asthma was 4.6% (0.1% for prenatal).

Conclusions: We conclude that the associations between both late-pregnancy prenatal exposure to antibiotics and postnatal exposure to antibiotics and an increased risk of asthma are plausible and consistent with a causal effect.

KEYWORDS
asthma, children, epidemiology, antibiotics, birth cohort



GRAPHICAL ABSTRACT

- Asthma in children was positively associated with prenatal and postnatal antibiotic prescriptions.
- These associations were observed after procedures to minimize bias and not observed with exposure to antibiotics before pregnancy.
- We conclude that these findings support causal effects in our target population.

1 | INTRODUCTION

There is evidence that children whose mothers received antibiotics during pregnancy (prenatal exposure), and children who were exposed to antibiotics in early life (postnatal exposure), are at higher risk of allergic diseases and asthma.[1–4] However, it is not clear to what extent this association is due to bias or is a causal association. Causal mechanisms are plausible: antibiotics taken by the mother in pregnancy and passed to her child, or taken by the child in early infancy, can lead to a Th-2 skewed immune response and asthma.[5,6] Given that most cases of asthma in the UK are allergic, it is plausible that changes in the immune system are a common ultimate proximal causal pathway shared by prenatal and postnatal antibiotic exposures. However, not all cases of asthma are allergic, and so, there can be different causal mechanisms in different asthma sub-types.[7] If it is a causal association, this is important from a public health perspective, given the large proportion of pregnant women and children who use antibiotics (33% and 70%, respectively, estimated for our target population), and the high prevalence of asthma in many settings.[7]

However, there is also evidence that this association with antibiotics can be due to unmeasured confounders. For example, maternal exposure to antibiotics before pregnancy,[8–10] and paternal exposure[9] have been reported as associated with asthma. Furthermore, it has been described that this association between prenatal and postnatal antibiotics and asthma decreased when assessed with sibling analysis that controls for shared familial and indoor environmental factors.[11]

In our observational study, we investigated whether prenatal and postnatal exposure to antibiotics had a causal association with an increased risk of asthma in children recruited to the Born in Bradford birth cohort study in England (BiB cohort).[12] We used several approaches to deal with different sources of bias and to assess causation (i.e., triangulation)[13]: maternal prescriptions 0–12 months before pregnancy were used as negative control exposure, the analysis was repeated excluding children with lower respiratory infection and/or asthma at 0–4 years to avoid reverse causation with postnatal antibiotic exposure, and sibling analysis was conducted to control for prenatal shared time-invariant maternal and environmental factors. We also assessed the heterogeneity of effects by mother's ethnicity and interaction between prenatal and postnatal exposures, and the disease burden.

Key messages

- Asthma in children was positively associated with prenatal and postnatal antibiotic prescriptions.
- These associations were observed after procedures to minimize bias and not observed with exposure to antibiotics before pregnancy.
- We conclude that these findings support causal effects in our target population.

2 | METHODS

The target population was 25,534 children born at the Bradford Royal Infirmary between 2007 and 2011, of which 13,858 (54%) were recruited to the BiB cohort.[12,14] We included all children of the BiB cohort except 173 children who died. The study population was the 13,685 children alive at the time of this study, from 12,248 mothers (1,384 mothers had >1 child). The complete case analyses with

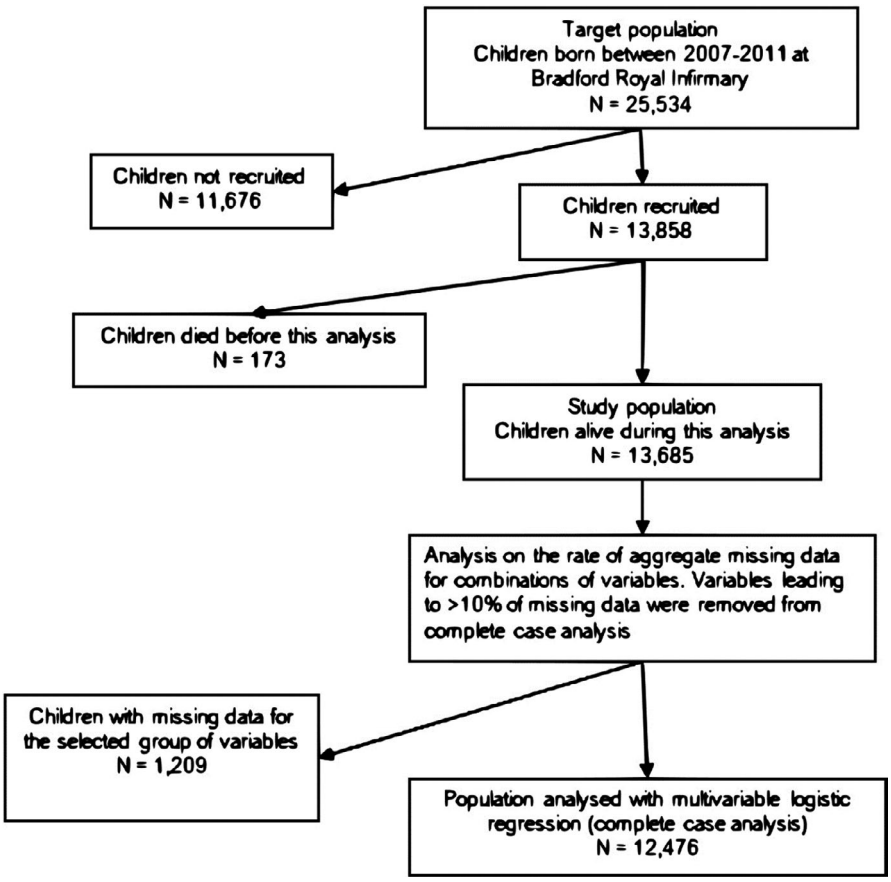


FIGURE 1 Derivation of the study population

multivariable logistic regression (MLR) was conducted with 12,476 children, of whom 2,622 were siblings (284 twins) (Figure 1). 2,826 of 12,476 were born by caesarean section, and the mothers of 89 children received intrapartum antibiotic prophylaxis to prevent post-surgical site infection. Every pregnant woman who attended Bradford Royal Infirmary antenatal clinic between 2007 and 2011 was invited to take part in the BiB cohort. Consenting women signed a consent form.

2.1 | Sources of data

Data on ethnicity, socio-economic and lifestyle factors were obtained from a baseline questionnaire administered at recruitment during pregnancy. Primary care clinical event and prescription data were obtained from linked electronic health records (EHR).[15] Obstetric and birth data were extracted from the hospital electronic maternity records, and data on children's body mass at 4–5 years were taken from the National Child Measurement Programme.

2.2 | Outcome definition

We conducted case-insensitive text mining of the EHR for the term "asthma". Of the 162 Read codes found (diagnostic codes that are used in primary care in the UK), we selected 148 to define asthma (Table S1), including Read codes validated in previous studies.[16] We used two case definitions. For the first, a child was a case if they had ≥1 of the selected Read codes from 5 to 8 years of age, as the diagnosis of asthma before 5 years old is difficult.[17] For the second definition, we searched for GP prescriptions for inhaled corticosteroids, bronchodilators and leukotriene receptor antagonists identified by text mining and British National Formulary (BNF) codes. We defined an asthma case if a child fulfilled the first definition and had ≥1 prescription for inhaled corticosteroids or leukotriene receptor antagonists between 5 and 8 years of age; this definition would have higher specificity than the first definition.

2.3 | Exposure

We searched the EHR for generic and common brand names of oral and systemic antibiotics (case-insensitive text mining), and BNF codes. We classified maternal exposure during pregnancy (prenatal exposure) as four dichotomous variables: first trimester (<93 days of gestational age); second trimester (93–184 days); third trimester until 28 days before the child's birth; and the period 7–27 days before the child's birth (see Table S2). Maternal prescriptions at 0–12 months before pregnancy were used as the negative control exposure, under the assumption it is associated with the same confounders that are

associated with maternal exposure during pregnancy, but with an implausible causal effect with asthma in the child.[18] Postnatal exposure was defined as a child's prescriptions of systemic antibiotics between 0 and 24 months old.

2.4 | Covariates

We first drew a directed acyclic graph with the main risk factors for asthma and then selected the variables related to the following: (1) the mother (history of asthma, eczema or hayfever, smoking, self-defined ethnicity, country of birth, age at child's birth, education, employment, diabetes during pregnancy, parity); (2) family size and (3) children (mode of delivery, obesity at 4–5 years old, prematurity (<37 weeks gestation), low birthweight (<2,500 g), gender, lower respiratory tract infection (LRTI) between birth and 24 months old (defined as EHR records with the terms "lower respiratory tract infection", "pneumonia", "syncytial infection" or "lower respiratory infection"). Maternal history of asthma and child's gender were always maintained in the regression models.

2.5 | Statistical analysis

Figure 2 shows the graphical representation. All the analyses applying the triangulation approach[13] described below were done for the 1st asthma definition and only the association analysis for the 2nd definition. Analyses were performed with Stata (Stata/SE 15 for Windows; StataCorp LP) and R statistical software (R Foundation).

Association analysis. First, we selected the variables that together in a regression resulted in $\lessapprox$10% of missing data. These variables were used in the complete case analysis (CCA) with MLR. Secondly, we conducted unadjusted associations between all variables in the CCA and the outcome (bivariable screening). Finally, all variables with unadjusted P value of $\lessapprox$0.25 were used to create five logistic regression models. Model 1 contained antibiotic exposure during pregnancy and other variables. In model 2, the negative control exposure was added to model 1. In model 3 (full adjusted model), postnatal exposure was added to model 2. In model 4, children with LRTI and/or asthma between 0 and 4 years were excluded from model 3 to avoid reverse causation with postnatal exposure. Model 5 (minimally adjusted model) was derived from model 3 after

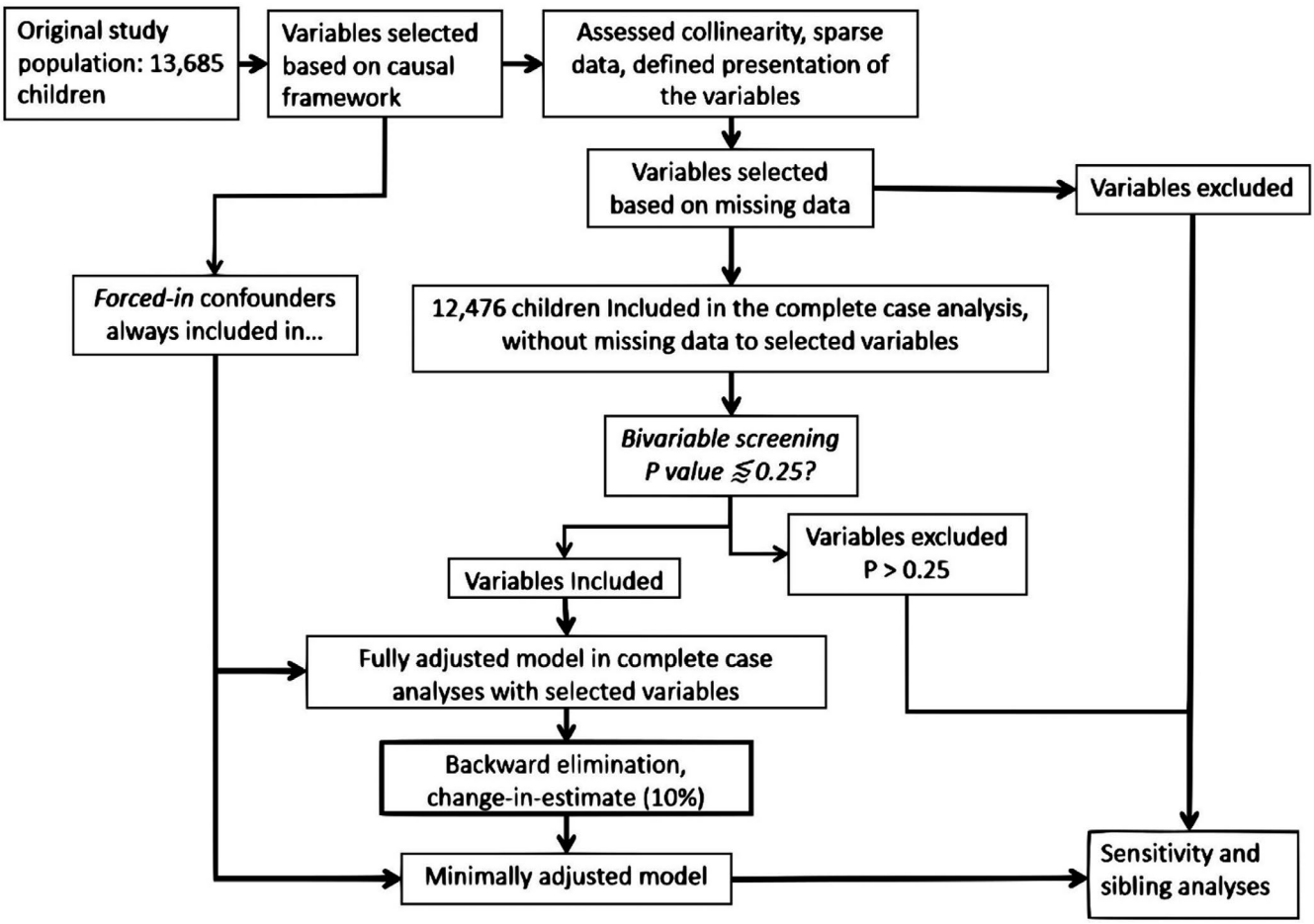


FIGURE 2 Strategy of analysis to assess associations between exposure to antibiotics and asthma. After the directed acyclic graph, we selected the available variables representing the factors with a strong association with the outcome and their proxies based on a hypothetical causal framework. The analysis started with an exploratory data analysis to describe missing data, extreme values, sparse data, conflicting data from different sources and multicollinearity

backward elimination (package "abe" in R),[19] based on change-in-estimate of odds ratio (OR) ≳10% and without using statistical significance. We also ran a matched analysis with conditional logistic regression with groups of siblings from the same mother, to control for shared time-invariant maternal and environmental factors.[20]

We estimated the E-value: "the minimum strength of association, on the risk ratio scale, that an unmeasured confounder would need to have with both the [exposure] and the outcome to decrease the association to the null (RR = 1)."[21] The interaction between prenatal and postnatal exposures, and heterogeneity of effects according to the mother's ethnicity (the distribution of the risk factors for asthma vary in relation to ethnicity[22]) and mode of delivery (caesarean vs. vaginal) were assessed with log-binomial regression due to the high prevalence of asthma. The risk of disease in the population due to the exposure (population attributable risk, PAR),[23] the population attributable fraction and the excess number of cases of asthma in the target population attributable to antibiotics were estimated for different hypothetical scenarios if the antibiotics had no effect on the asthma cases (Stata commands *punaf* and *regpar*).[24]

We conducted sensitivity analyses to assess the following: ascertainment bias (stratification by the total number of days with GP visits or to the clinics a child had between 0 and 24 months old, excluding days with records on asthma, wheeze and LRTI); clustering effect (robust variance estimator, mothers as clusters); effect of each variable excluded from the MLR (missing data or bivariable screening); and effect of breastfeeding, positive skin prick test, child care and pets, with data available in subgroups of the BiB cohort[22,25] (unmeasured confounders).[26,27]

3 | RESULTS

The CCAs were conducted on 12,476 children (Table 1). Differences were observed between CCA and 151 children with missing data, especially in relation to lower proportion of children whose mothers were born in the UK, mothers not employed and higher maternal education. In the CCA, 10.6% (n = 1,322) of children had asthma, 46.3% (n = 5,774) had mothers of Pakistani heritage, and 46.1% (n = 5,746) had mothers born outside the UK.

3.1 | Initial data analysis

In the 12,476 children, several factors were associated with an increased risk of asthma, in addition to maternal and postnatal exposure to antibiotics (Table S3): they were related to children (male, born by caesarean section, low birthweight, prematurity, obesity at 4 years old and LRTI in infancy) and to the mother and family (Pakistani origin, history of allergic diseases, not born in the UK, not being employed at baseline and as number of people in the household increased).

3.2 | Association between exposure to antibiotics and childhood asthma

In the unadjusted association, the risk of asthma was higher among children whose mothers received antibiotics at 0–12 months before pregnancy (negative control exposure) and in each trimester of pregnancy, and among children exposed to antibiotics at 0–24 months old (Table 2). However, in the fully adjusted model (model 3), the ORs decreased and became closer to 1 (95% CI 0.9, 1.10), except for exposure during pregnancy at 7–27 days before birth, OR = 1.40 (1.05, 1.87), and at 0–24 months old (postnatal exposure), OR = 2.00 (1.71, 2.34) (Table 2). When children with previous lower respiratory disease were excluded (model 4), the OR decreased, but the association was still evident for the exposure at 0–24 months old, OR = 1.67 (1.39, 2.01). Results for the second definition are presented in Table S4. These associations were observed for all classes of antibiotics, but the small numbers did not allow further comparisons between these different classes (Table 3).

The risk of asthma increased as the number of prescriptions of any antibiotics at 0–24 months increased, however with overlapping confidence intervals (Table 4). Analysis of dose-response with exposure during pregnancy at 7–27 days before birth was not conducted due to small numbers.

Adjustment for clustering effect and the variables excluded from the CCA changed the OR between −0.7% and +15% in comparison with the fully adjusted model (model 3 in Table 2). The exception was with the association with childhood exposure at 0–24 months old after the adjustment for previous GP consultations: the OR was 2.00 (1.71, 2.34) in model 3 and 1.55 (1.32, 1.82) when adjusted for GP consultations.

Analysis of siblings. For maternal exposure at 7–27 days before the birth, there were 11 groups of siblings with 2 children (22 children) and 1 group with 3 children; for exposure of the children at 0–24 months old, there were 172 groups of siblings with 2 children (344 children) and 4 groups with 3 children. The estimates were adjusted for variables not shared by the siblings (child's gender, mode of delivery, child obesity at 4 years, low birthweight and maternal age at birth). There was a higher risk of asthma among those children exposed at 0–24 months with OR = 1.99 (1.00, 3.93) (Table S5). Results for maternal exposure had small numbers of children and therefore were difficult to interpret.

3.3 | Sensitivity bias analysis

The adjusted risk ratio (Table S6) varied from 1.31 to 1.47 for prenatal exposure and from 1.71 to 1.89 for postnatal exposure. The biggest percent change-in-estimate was with skin prick test (SPT) and for prenatal exposure: the relative risk would be >+10.2% with adjustment (from 1.32, 1.47). There were no remarkable changes with child care, breastfeeding and presence of pets.

TABLE 1 Characteristics of the children separately for the study and analysed populations

Characteristics of the children and their mothers	Study population, *N* = 13,685		Children analysed[a]
	All children		
	With valid data	Missing	*N* = 12,476
Child defined as asthma case at 5–8 years old	1,406 (10.4)	151	1,322 (10.6)
Maternal antibiotic prescription:			
0–12 months before pregnancy[b]	4,735 (35.1)	190	4,531 (36.3)
1st pregnancy trimester	2,079 (15.4)	190	1,994 (16.0)
2nd pregnancy trimester	2,130 (15.8)	190	2,033 (16.3)
3rd trimester (≥28 days before the birth, range)[c]	1,392 (10.3)	190	1,336 (10.7)
3rd trimester (7–27 days before the birth)[c]	425 (3.1)	190	392 (3.1)
All period of pregnancy (1st to 3rd trimester)	4,579 (33.5)	190	4,384 (35.1)
Child had antibiotic prescriptions at 0–24 months old	9,459 (70.7)	298	8,872 (71.1)
Child's gender (male)	7,063 (51.6)	0	6,404 (51.3)
Mother with history of asthma	2,202 (16.3)	152	2,045 (16.4)
Mother with history of atopic eczema	3,718 (27.5)	152	3,508 (28.1)
Mother with history of hayfever	2,965 (21.9)	152	2,768 (22.2)
Mother with history of smoking:			
During 1st pregnancy trimester	1,076 (7.9)	14	1,012 (8.1)
During 2nd pregnancy trimester, 3rd or both	635 (4.6)	14	581 (4.7)
During 0–24 months of child's life	2,545 (18.6)	14	2,340 (18.8)
Child with obesity at 4–5 years	1,206 (9.0)	232	1,133 (9.1)
Child with mother of Pakistani ethnicity (vs. others)	6,029 (45.4)	406	5,774 (46.3)
Mother with diabetes during pregnancy[d]	150 (1.1)	233	143 (1.1)
Mother born in the UK	7,185 (63.4)	2,361	6,730 (53.9)
Child born by caesarean section	3,058 (22.9)	355	2,826 (22.7)
Child had lower respiratory infection at 0–24 months old[e]	293 (2.2)	155	270 (2.2)
Child born at <37 weeks of gestational age	842 (6.3)	322	764 (6.1)
Mother not employed at baseline	6,306 (55.8)	2,374	5,925 (47.5)
Maternal education higher than A level at baseline	2,889 (27.7)	3,262	2,651 (21.2)
Child with low birthweight (<2,500g)	1,112 (8.3)	323	1,033 (8.3)
Parity at child's birth[f]			
None	5,099 (39.6)	814	4,730 (37.9)
One	3,732 (29.0)	814	3,478 (27.9)
Two or more	4,040 (31.4)	814	3,803 (31.7)
Children with siblings[g]	2,821 (20.6)	0	2,551 (30.5)
Number of people in household at baseline, mean (SD)	4.1 (2.3)	2,370	4.1 (2.3)
Maternal age at child birth (years), mean (SD)	27.5 (5.6)	0	27.6 (5.6)

[a]Children included in the complete case analysis (CCA) with multivariable logistic regression.

[b]Used as negative control exposure.

[c]Among the 12,476 children, 1,336 mothers had prescriptions of antibiotics ≥28 days before the birth, 392 mothers 7–27 days before the birth and (not in the table) 92 in both periods. The gestational age of birth for the 1,336 children was between 227 and 313 days, and between 197 and 292 days for the 392 children.

[d]Mother with prescriptions of insulin or other relevant drugs during pregnancy period, or ≥1 Read code for diabetes. It could be diabetes developed during pregnancy or pre-existent

[e]Records with Read codes with the terms "lower respiratory tract infection", "pneumonia", "syncytial infection" or "lower respiratory infection" at 0–24 months old.

[f]Number of previous parities identified by birth certification.

[g]Number of children whose mothers had >1 child in the study population or analysed population.

TABLE 2 Association between exposure and other variables vs asthma (based only on Read codes between 5 and 8 yo) in different models adjusted for the study variables shown in the table

Study variables	Unadjusted *N* = 12,476	Model 1 *N* = 12,476	Model 2 *N* = 12,476	Model 3 *N* = 12,476	Model 4[a] *N* = 11,560	Model 5[b] *N* = 12,476
Maternal antibiotic prescriptions during:						
0–12 months before pregnancy, negative control	1.19 (1.06, 1.33)	Not used	1.03 (0.91, 1.17)	0.99 (0.88, 1.12)	0.98 (0.84, 1.15)	-
First pregnancy trimester	1.29 (1.11, 1.49)	1.14 (0.98, 1.33)	1.14 (0.97, 1.32)	1.10 (0.95, 1.29)	1.18 (0.97, 1.43)	-
Second pregnancy trimester	1.29 (1.11, 1.49)	1.13 (0.97, 1.31)	1.12 (0.96, 1.31)	1.10 (0.94, 1.28)	1.18 (0.98, 1.43)	-
Third trimester (≥28 days before the birth)	1.09 (0.91, 1.31)	0.96 (0.79, 1.15)	0.95 (0.79, 1.15)	0.93 (0.77, 1.12)	0.96 (0.76, 1.22)	-
Third trimester (7–27 days before the birth)	1.58 (1.20, 2.09)	1.43 (1.07, 1.91)	1.43 (1.07, 1.90)	1.40 (1.05, 1.87)	1.15 (0.77, 1.70)	1.44 (1.08, 1.91)
Standard error	0.22536	0.20969	0.20913	0.20616	0.23106	0.20820
Child with antibiotic prescriptions at 0–24 months (Yes)	2.32 (1.99, 2.70)	Not used	Not used	2.00 (1.71, 2.34)	1.67 (1.39, 2.01)	2.22 (1.90, 2.58)
Standard error	0.18066	-	-	0.15966	0.15829	0.17370
Child's gender	1.56 (1.39, 1.75)	1.58 (1.41, 1.78)	1.58 (1.41, 1.78)	1.53 (1.71, 2.34)	1.45 (1.24, 1.68)	1.52 (1.35, 1.71)
Maternal history of asthma	1.93 (1.69, 2.20)	1.83 (1.59, 2.11)	1.83 (1.58, 2.11)	1.81 (1.36, 1.73)	1.86 (1.55, 2.23)	1.89 (1.65, 2.17)
Maternal history of eczema	1.28 (1.13, 1.45)	1.13 (1.00, 1.29)	1.13 (1.00, 1.29)	1.12 (0.99, 1.28)	1.13 (0.96, 1.33)	-
Maternal history of hayfever	1.61 (1.42, 1.83)	1.36 (1.20, 1.56)	1.36 (1.19, 1.56)	1.34 (1.18, 1.53)	1.20 (1.01, 1.43)	-
Child's obesity at 4–5 yo	1.51 (1.27, 1.80)	1.46 (1.22, 1.75)	1.46 (1.22, 1.75)	1.44 (1.18, 1.53)	1.38 (1.09, 1.74)	-
Child with mother of Pakistani ethnicity	1.40 (1.25, 1.57)	1.44 (1.28, 1.63)	1.44 (1.28, 1.62)	1.38 (1.20, 1.72)	1.27 (1.09, 1.48)	-
Mode of delivery (caesarean)	1.20 (1.05, 1.37)	1.16 (1.01, 1.33)	1.16 (1.01, 1.33)	1.15 (1.22, 1.55)	1.06 (0.89, 1.27)	-
Child with lower respiratory infection at 0–24 months (Yes)[d]	2.88 (2.17, 3.82)	2.63 (1.97, 3.52)	2.63 (1.97, 3.51)	2.29 (1.71, 3.06)	Not used	-
Gestational age at birth (<37 weeks)	1.39 (1.12, 1.72)	1.15 (0.88, 1.50)	1.15 (0.88, 1.49)	1.15 (0.88, 1.50)	1.13 (0.80, 1.59)	-
Low birthweight (<2,500 g)	1.37 (1.14, 1.66)	1.23 (0.97, 1.55)	1.23 (0.97, 1.56)	1.23 (0.97, 1.55)	1.21 (0.89, 1.63)	-

Note: Model 1: variables relating to antibiotic exposure during pregnancy and confounders. Model 1 included all variables listed in the table, except when is written "Not used" (negative control exposure and child with antibiotic prescriptions at 0–24 months of life). In model 2, the negative control exposure was added to model 1. In model 3, postnatal exposure was added to model 2. In model 4, children with lower respiratory infection and/or asthma between 0 and 4 years were excluded from model 3 to avoid reverse causation with postnatal exposure. Model 5 (minimally adjusted model) was derived from model 3 after backward elimination.

[a]similar to model 3 but excluding those children with Read codes for asthma between 0 and 47 months of life (0–3 yo) and for lower respiratory infection at 0–24 months of life.

[b]This model was selected by using package "abe" in R.(David W. Hosmer et al., 2013, Blagus, 2017), with all variables in model 3, maintaining the two exposures and a priori confounders in the regressions, with no interaction terms, and selection criteria based on change-in-estimate of >10% (statistical significance not used).

TABLE 3 Association between systemic antibiotic prescriptions and asthma (based only on Read codes between 5 and 8 yo) among 12,476 children, separately for prenatal or postnatal exposure, and class of antibiotics

Systemic antibiotic prescriptions	*N*	Asthma case *n* (%)	Odds ratio adjusted (95% C.I.)	
			For one class of antibiotics [a]	For all classes of antibiotics[b]
To the mother 7–27 days before birth [c]				
Penicillins				
No	12,251	1,283 (10.5)	1	1
Yes	225	39 (17.3)	1.71 (1.20, 2.44)	1.71 (1.20, 2.44)
Cephalosporins				
No	12,327	1,301 (10.6)	1	1
Yes	149	21 (14.1)	1.31 (0.82, 2.10)	1.30 (0.81, 2.08)
Others				
No	12,442	1,319 (10.6)	1	1
Yes	34	3 (8.8)	0.79 (0.39, 2.60)	0.70 (0.21, 2.33)
To the child at 0–24 months old				
Penicillins				
No	4,042	264 (6.5)	1	1
Yes	8,434	1,058 (12.5)	1.97 (1.71, 2.27)	1.72 (1.49, 1.99)
Cephalosporins				
No	11,565	1,157 (10.0)	1	1
Yes	911	165 (18.1)	1.91 (1.59, 2.28)	1.49 (1.24, 1.79)
Macrolides				
No	10,044	912 (9.1)	1	1
Yes	2,432	410 (16.9)	1.94 (1.71, 2.20)	1.67 (1.46, 1.90)
Others				
No	12,207	1,279 (10.5)	1	1
Yes	269	43 (16.0)	1.68 (1.20, 2.34)	1.47 (1.05, 2.07)

[a]Each class of antibiotics each time and also adjusted for maternal history of asthma and child's gender.

[b]With the variables for all classes of antibiotics together in the regression, and adjusted for maternal history of asthma and child's gender.

[c]The variable on macrolides was excluded because there were no asthma cases among the exposed.

TABLE 4 Percentage of asthma cases (based only on Read codes between 5 and 8 yo) at 5–8 years old and the 95% confidence intervals, separately for number of prescriptions of any antibiotic to the children at 0–24 months old, adjusted for maternal history of asthma and child's gender. *N* = 12,476

Number of prescriptions	Asthma cases n/N (%)	% Adjusted (95% C.I.)
0	210/3604 (5.8)	6.0 (5.2, 6.8)
1	233/2842 (8.2)	8.3 (7.3, 9.3)
2	213/1945 (11.0)	10.9 (9.6, 12.3)
3	165/1368 (12.1)	11.9 (10.2, 13.6)
4	119/816 (14.6)	14.1 (11.8, 16.5)
5	96/588 (16.3)	16.0 (13.1, 18.9)
6	72/383 (18.8)	18.3 (14.5, 22.1)
7	62/295 (21.0)	20.2 (15.7, 24.7)
≥ 8	152/635 (23.9)	22.6 (19.5, 25.8)
Total	1,322/12,476 (10.6)	10.6 (10.1, 11.1)

3.4 | E-value

For the association with exposure at 7–27 days before birth, the observed OR was 1.40 and the E-value was RR = 2.15. Therefore, if the true value of the association were 1.0, an unmeasured confounder would need to have a RR of at least 2.15 with both exposure and asthma to produce the observed OR = 1.40. For postnatal exposure with OR = 1.67, the E-value was 2.73.

3.5 | Heterogeneity of effect/interaction

The children who were exposed at 7–27 days before birth (prenatal) and at 0–24 months of life (postnatal) had a higher risk of disease (16.7%) in comparison with those with only prenatal exposure (10.5%) or only postnatal exposure (12.4%). The effect of exposure (RR) was lower among children whose mothers were of Pakistani ethnicity (prenatal: 1.31 (0.94; 1.81), postnatal: 1.83 (1.49; 2.24)) in

comparison with other ethnicities (prenatal: 1.53 (1.10; 2.14), postnatal: 2.13 (1.75; 2.60)) and higher among children born by caesarean section (prenatal: 1.68 (1.13; 2.51), postnatal: 2.19 (1.63; 2.93)), in comparison with vaginal delivery (prenatal: 1.31 (0.99; 1.75), postnatal: 2.01 (1.71; 2.36)). All results had $p > .25$ for the interaction term.

3.6 | Impact

Among the 12,476 children, 3.1% of the mothers received antibiotics during pregnancy at 7–27 days before birth, and the excess number of asthma cases due to this exposure was estimated as 16 cases (33 cases in the target population, 25,534, PAR = 0.1%, PAF = 1.2%) (scenario 1 in Table S7). 70% of children received antibiotics at 0–24 months old, and the excess number of cases was 569 cases (1,164 cases in 25,534, PAR = 4.6%, PAF = 43.0%) (scenario 2).

4 | DISCUSSION

The two exposure variables, maternal antibiotics prescriptions at 7–27 days before the child's birth and prescriptions for the child at 0–24 months, were associated with an increased risk of asthma, even after assessment for different biases: adjustment for relevant potential confounders, confounding by indication (LRTI), sibling analysis, reverse causation (postnatal exposure), sensitivity analysis for unmeasured confounders and ascertainment bias. There was no association with maternal antibiotic prescriptions before pregnancy, and so, the association was restricted to when a causal pathway was plausible. There was an increase in the risk of asthma with the increase in the number of prescriptions (monotonic trend). The E-value was estimated as 2.15 and 2.73 (risk ratio scale). The results on heterogeneity of effects were considered inconclusive.

The association between antibiotic exposure and asthma has been observed previously: a literature review and meta-analyses by Zhao et al,[28] which included papers from a previous review, showed a pooled OR of 1.22 (1.02, 1.45) for the whole pregnancy period (10 studies) but with high heterogeneity (I^2 statistics = 90%), and so, the pooled OR is difficult to interpret. For the third trimester, it was 1.33 (1.11, 1.60) (2 studies, I^2 = 0%). Other studies published since this review also found an association,[4,9,29–31] with exceptions.[32] Evidence of dose-response has also been reported.[10]

In previous studies, similar associations were observed for antibiotic exposure during, before and after pregnancy,[8,10] and the association decreased with sibling analysis.[11] These findings are in conflict with the causal hypothesis. However, in our study population, neither antibiotics given to the mother before pregnancy was associated with asthma nor did the siblings' analysis decrease or change the direction of the association. Therefore, in our study population the results were not conflicting.

The fact that the association was not observed before pregnancy gives support to specificity of the exposure during pregnancy.[33] Causal mechanisms have been suggested as biologically plausible: prenatal exposure to antibiotics by the mother and in early infancy could change the gut microbiome of the child and ultimately can lead to asthma.[5] Alternatively, but not mutually exclusive, antibiotics can change the maternal microbiota, which are transmitted to the child and could lead to changes in the child's immune response.[34] The diversity of the gut and lung microbiota of children would be disrupted, and reduced diversity and increased proportions of some specific pathogenic species have been associated with the development and severity of asthma.[5,6,35,36] A similar process would be involved in the higher risk of asthma among children born by caesarean section,[37] and antibiotics and obesity in children.[38] Therefore, there is evidence of this causal mechanism from other events. Heterogeneity of effects and "causal interaction" can be important to identify subgroups in which a hypothetical intervention would result in a larger effect and to shed light on an explanation for the causal effect.[39] In the BiB cohort, the proportion of maternal smoking during pregnancy is higher in White British mothers than in mothers of Pakistani ethnicity.[22] However, it was not possible to reach a conclusion because of the high p value. We conclude that the associations between both late-pregnancy prenatal exposure to antibiotics and postnatal exposure to antibiotics and an increased risk of asthma are plausible and consistent with a causal effect.

The strengths of this study are a large sample size, with detailed demographic data, and follow-up data through linkage with primary care data. Several relevant potential confounders were included such as indicators of heredity and respiratory infection in infancy. This study had a well-defined target population, which is important to make causal effects meaningful.[40] Instead of trying to address our causal question based simply on a single regression model, we used triangulation.[13] The variables representing maternal antibiotic exposure during pregnancy were defined after looking at the data, which could be considered a reason for the cautious interpretation of their associations with asthma.

Unmeasured confounding remains a possibility, and it is a potential limitation in an observational study.[41] Relevant data such as on indoor environmental factors and breastfeeding were only available for sub-group of the study population, and thus, we conducted a bias analysis, but it does not replace an adjustment with the variables. If the magnitude of association is high, it is less likely to be explained by unmeasured confounders.[42] We took the E-value as an indicator for the magnitude of association for this hypothetical confounding bias, by assuming that there would be one confounder that was not adjusted for, a strong assumption.[43] Unmeasured confounders with magnitude of association with asthma could be higher than 2.0[44]; therefore, confounding by an unmeasured confounder is plausible.

Evidence supporting a causal association is needed before proposing public health interventions and changes in clinical practice. The decision-making process in public health ultimately is a subjective judgement considering our expectations of the benefits and harms based on evidence from different sources and including our current knowledge.[45,46] In our study and target population, the attributable excess number of cases would be small for prenatal exposure at 7–27 days before the birth, but this would be much higher for

postnatal exposure in early childhood, supporting the potential benefits if exposure to antibiotics is reduced. As a caveat, the interpretation of the excess number attributable to the exposure depends on whether the association is causal and whether elimination of the exposure has no effect on the distribution of other risk factors.[47] Furthermore, the excess number attributable to the exposure is not equal to the number of cases caused by the exposure.[48]

Evidence of any exposure-disease association depends also on the frequency of the different causes, and asthma results from the combination of several factors, which may not have the same distributions in different populations. Therefore, evidence of causation from a specific population is not necessarily generalizable to another population, consistency does not necessarily support causation,[40] and inconsistency in different studies is not necessarily due to methodological issues. If there is good evidence of a causal association, whether the magnitude of the effect would justify a change in recommendations and practice should be contextualized for different populations, considering the trade-offs between the potential harms and benefits, and feasibility of reducing the consumption of antibiotics in infancy. For example, the decision to administer antibiotic prophylaxis to mothers during caesarean before the surgical cut to prevent surgical site infection should be balanced with the potential of long-term harm to their children's health.[49] This more pragmatic approach should be considered, instead of trying to generalize the findings or wait for ultimate proof of a causal association or consistency of results from different settings. If, in a specific population, there is fair evidence of a significant effect from a public health perspective, and it is feasible to reduce the use of antibiotics, an intervention may be justified. Furthermore, these findings support the policy that seeks to minimize the usage of antibiotics in children,[50] given the potential considerable numbers if our results were extrapolated to the UK population. In our target population, it would also be prudent to be judicious in the use of antibiotics in the last month of pregnancy.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

SSC and LP conceived this investigation and wrote the manuscript. SSC executed analyses. All authors critically revised the manuscript for intellectual content, interpreted study findings, and read and approved the final manuscript.

ETHICAL APPROVAL

Born in Bradford cohort study has Bradford Research Ethics Committee approval (Ref: 07/H1302/112). The NIHR antibiotics study has Health Research Authority (HRA) and Health and Care Research Wales (HCRW) approval (ref: 238908).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author (https://borninbradford.nhs.uk/research/how-to-access-data/). The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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