Prenatal antibiotic exposure and childhood asthma: a population-based study

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Maternal antibiotic use is associated with childhood asthma, but the association is not specific to antibiotic use during pregnancy http://ow.ly/G5j230jAzs5

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ABSTRACT Antibiotic use during infancy alters gut microbiota and immune development and is associated with an increased risk of childhood asthma. The impact of prenatal antibiotic exposure is unclear. We sought to characterise the association between prenatal antibiotic exposure and childhood asthma.

We performed a population-based cohort study using prescription records, hospitalisation records and physician billing claims from 213 661 mother–child dyads born in Manitoba, Canada between 1996 and 2012. Associations were determined using Cox regression, adjusting for maternal asthma, postnatal antibiotics and other potential confounders. Sensitivity analyses evaluated maternal antibiotic use before and after pregnancy.

36.8% of children were exposed prenatally to antibiotics and 10.1% developed asthma. Prenatal antibiotic exposure was associated with an increased risk of asthma (adjusted hazard ratio (aHR) 1.23, 95% CI 1.20–1.27). There was an apparent dose response (aHR 1.15, 95% CI 1.11–1.18 for one course; aHR 1.26, 95% CI 1.21–1.32 for two courses; and aHR 1.51, 95% CI 1.44–1.59 for three or more courses). Maternal antibiotic use during 9 months before pregnancy (aHR 1.27, 95% CI 1.24–1.31) and 9 months postpartum (aHR 1.32, 95% CI 1.28–1.36) were similarly associated with asthma.

Prenatal antibiotic exposure was associated with a dose-dependent increase in asthma risk. However, similar associations were observed for maternal antibiotic use before and after pregnancy, suggesting the association is either not directly causal, or not specific to pregnancy.

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Introduction

Asthma is the most common chronic disease of childhood [1], affecting >10% of children worldwide [2]. In the United States, the total cost of asthma to society is estimated at USD 56 billion per year [3]. Similarly, in Canada asthma is a leading cause of healthcare utilisation, work absenteeism, lost productivity and diminished quality of life [4]. Given this large clinical and economic burden, and because there is no cure for asthma, it is important to identify modifiable risk factors to inform asthma prevention strategies.

The US Centers for Disease Control and Prevention report that antibiotics are prescribed at 12.6% of all ambulatory care visits, and 30% of these prescriptions may be unnecessary [5]. Across different settings, 20–40% of females are prescribed antibiotics during pregnancy [6–9], accounting for nearly 80% of all drugs used by pregnant females [6]. Prenatal antibiotic use may result in fetal exposure, as at least 11 types of broad-spectrum antibiotics cross the placenta, including penicillins, tetracyclines and lincosamides [10]. Mounting evidence shows that early-life exposure to antibiotics can have long-term health effects by perturbing the gut microbiota and disrupting immune system development [11–13]. However, relatively few of these studies have addressed antibiotic use during pregnancy, which could modify the maternal microbiota before its transmission to the fetus or infant during gestation, delivery, postnatal contact and lactation [14, 15].

Several longitudinal studies [16–18] and meta-analyses [19–21] indicate that antibiotic exposure during infancy is a risk factor for asthma (pooled OR 1.52, 95% CI 1.30–1.77; 20 studies, n=685 550) [20], although some reports suggest confounding by indication or reverse causation [21, 22]. Fewer studies have evaluated prenatal antibiotic exposure [23], with some [24–28] reporting increased asthma risk and others finding no association [29, 30]. These studies have variably reported associations by antibiotic type [26, 27, 29], dose [26, 28] and trimester of exposure [27–29], but no single study has addressed all of these factors, and only a few have accounted for postnatal antibiotic use [25, 31]. A recent study by Stokholm *et al.* [30] found that maternal antibiotic use before, during and after pregnancy was similarly associated with asthma risk among offspring, suggesting that maternal propensity for infection (rather than antibiotic use *per se*) may be responsible for this association.

Using administrative health data capturing all children born in Manitoba, Canada from 1996 to 2012, we undertook a population-based study examining the association of maternal antibiotic use and childhood asthma. We classified antibiotics by type, number of courses and timing of exposure, and controlled for postnatal antibiotic use. In addition, we performed a sensitivity analysis to determine whether associations were specific to maternal antibiotic use during pregnancy.

Patients and methods

Study design, population and data sources

We conducted a retrospective cohort study of mother–infant dyads in Manitoba, Canada. Using Manitoba's health administrative data housed at the Manitoba Centre for Health Policy [32], we created a provincial birth cohort comprising all children born in Manitoba between 1996 and 2012. Data sources included physician billing claims, hospitalisation discharge abstracts and drug prescriptions collected by the Manitoba Health Services Insurance Plan (MHSIP) and the Drug Program Information Network (DPIN). The MHSIP and DPIN databases are reliable and valid data sources [33, 34]. Database record linkages were achieved through anonymised personal identifiers and a family registration number permitted linkage of maternal and child records. We included all dyads where linked maternal and child records were available, the mother was continuously registered with MHSIP for ⩾1 year before and ⩾1 year after pregnancy, and the child was continuously registered for ⩾3 years after birth (n=213 661 linked dyads from 235 891 total eligible births; 90.6%). Children without linked maternal data were less likely to develop asthma (incidence rate 8.74 *versus* 10.16 per 1000 person-years). This study was approved by the health research ethics board at the University of Manitoba and the Health Information Privacy Committee.

Main exposure: maternal antibiotic use during pregnancy

Maternal antibiotic use was determined from records of oral antibiotic prescriptions filled at community pharmacies and classified by dose (number of prescribed courses), timing (trimester of pregnancy, calculated from the infant's date of birth and accounting for gestational age) and type of antibiotic. All oral antibiotics were considered; they were grouped according to the Anatomical Therapeutic Chemical (ATC) classification system as follows: β-lactam penicillins (J01C); other β-lactams (J01D); macrolides, streptogramins and lincosamides (J01F); sulphonamides and trimethoprim (J01E); and tetracyclines (J01A), quinolones (J01M) and others (J01B, J01G, J01X). Antibiotics dispensed or administered in hospital are not captured in the DPIN database.

Primary outcome: child asthma

Asthma was defined as meeting any of the following criteria after the age of 5 years: 1) any hospitalisation for asthma; 2) two or more physician diagnoses of asthma, ⩾3 months apart and within a 1-year period; or 3) two or more prescriptions for asthma medications within a 1-year period. The age requirement was applied because 5 years is the minimum age for confirming asthma diagnosis by lung function testing, and misdiagnosis is common before this age [35]. We used the index date (the date when the child first met any of the above criteria, which could be prior to age 5 years) to capture incident cases for survival analysis. This definition was based on the validated definition applied by Kozyrskyj *et al.* [17] using the same administrative database. We modified this definition to increase specificity by requiring repeated physician diagnoses to be ⩾3 months apart.

Potential confounders

The following potential confounders were documented from administrative health records: infant sex, residence location (urban or rural), length of gestation, number of siblings and maternal asthma (defined using the algorithm described earlier). Postnatal antibiotic exposure in the first year of life (any or none) was determined from infant prescription records.

Statistical analysis

We conducted a time-to-event analysis, measuring time to event from a child's birthdate to the earliest of the following dates: date the child first met the asthma diagnosis definition, death, loss to follow-up or the end of the study period (March 31, 2015). Associations between prenatal antibiotic exposure and

TABLE 1 Characteristics of study population: mother–infant dyads in Manitoba, Canada, 1996–2012

Dyads n	213 661
Maternal age years	27.6±5.9
Infant birthweight g	3493±619
Gestational age weeks	39.1±1.9
Sex	
Male	109 437 (51.2)
Female	104 224 (48.8)
Delivery method	
Caesarean section	41 248 (19.3)
Vaginal	172 413 (80.7)
Feeding method at birth	
Fully breastfed	108 147 (50.6)
Partially breastfed	60 891 (28.5)
Formula-fed	41 026 (19.2)
Unknown	3597 (1.7)
Residence location	
Urban	115 604 (54.1)
Rural	97 549 (45.7)
Unknown	508 (0.2)
Number of children in household	
1	80 939 (37.9)
2	71 403 (33.4)
3	34 234 (16.0)
⩾4	26 842 (12.6)
Unknown	243 (0.1)
Maternal asthma	
Yes	12 780 (6.0)
No	200 881 (94.0)
Income quintile	
Q1 (lowest)	56 019 (26.2)
Q2	44 214 (20.7)
Q3	39 797 (18.6)
Q4	39 017 (18.3)
Q5 (highest)	34 106 (16.0)
Unknown	508 (0.2)

Data are presented as mean±SD, or n (%), unless otherwise stated.

childhood asthma were estimated using Cox regression models and reported as crude and adjusted hazard ratios (HR and aHR, respectively) and 95% confidence intervals, with adjustment for known asthma risk factors (*e.g.* male sex, maternal asthma) and confounders that either changed the crude estimates appreciably or were considered confounders *a priori* (*e.g.* antibiotic use in infancy, socioeconomic status). We conducted sensitivity analyses examining maternal antibiotic use during the 9-month window before and after pregnancy (defined based on the infant's birth date and gestational age). We modelled interaction terms to test for effect modification by infant sex, method of birth and newborn feeding method, and tested for the significance of including interaction terms using likelihood ratio tests.

Results

Our study population consisted of 213 661 mother–child dyads with a median follow-up time of 9.3 years from birth. The mean maternal age was 27.6±5.9 years and 6.0% of mothers had asthma (table 1). The majority (54.1%) lived in urban settings and 37.9% of children were first-born. Overall, 36.8% of mothers received antibiotics during pregnancy, 45.2% of infants received antibiotics in their first year of life, and 10.1% of children developed asthma (incidence rate 10.2 per 1000 person-years) (tables 2 and 3). Maternal antibiotic use varied slightly by trimester, from 16.2% in the first trimester to 18.4% in the second trimester and 14.7% in the third trimester. The majority of mothers receiving antibiotics were prescribed a single course (22.1% of all mothers) while fewer received two (8.4%) or more (6.2%) courses during their pregnancy. β-lactam penicillins were the most commonly prescribed type of antibiotic (24.6%), with fewer mothers receiving other β-lactams (6.1%); macrolides, lincosamides or streptogramins (7.1%); tetracyclines, aminoglycosides or quinolones (7.5%); and sulphonamides or trimethoprim (2.6%).

Mothers with asthma were more likely to use antibiotics during pregnancy (56.2% *versus* 35.5% among mothers with *versus* without asthma, p<0.0001), and their children had a higher rate of asthma (21.0 *versus* 9.6 cases per 1000 person-years, p<0.0001) (table 3). Preterm birth and infant antibiotic use were positively associated with increased maternal antibiotic use and child asthma. In contrast, rural residence location and higher birth order were both positively associated with maternal antibiotic use, but inversely associated with child asthma. These potential confounders were included in multivariable models to determine the independent association of maternal antibiotic use and child asthma (table 4).

Children born to mothers receiving antibiotics during pregnancy had significantly higher rates of asthma (11.9 per 1000 person-years, 95% CI 11.6–12.1) compared to their unexposed counterparts (9.2 per 1000 person-years, 95% CI 9.0–9.4) (table 3) (HR 1.29, 95% CI 1.26–1.33). This association persisted

TABLE 2 Maternal and infant antibiotic use in Manitoba, Canada, 1996–2012

Dyads n	213 661
Any maternal antibiotic use during pregnancy	
No	135 139 (63.2)
Yes	78 522 (36.8)
Number of maternal antibiotic courses during pregnancy	
0	135 139 (63.2)
1	47 286 (22.1)
2	17 954 (8.4)
⩾3	13 282 (6.2)
Type of maternal antibiotics used during pregnancy	
β-lactams, penicillins	52 598 (24.6)
β-lactams, others	12 998 (6.1)
Macrolides, streptogramins, lincosamides	15 120 (7.1)
Tetracyclines, quinolones, others	16 074 (7.5)
Sulphonamides and trimethoprim	5615 (2.6)
Timing of maternal antibiotic use	
9 months before pregnancy	75 166 (35.2)
Pregnancy trimester 1	34 562 (16.2)
Pregnancy trimester 2	39 340 (18.4)
Pregnancy trimester 3	31 330 (14.7)
9 months after pregnancy	75 761 (35.5)
Any infant antibiotic use before 12 months	
No	117 136 (54.8)
Yes	96 525 (45.2)

Data are presented as n (%), unless otherwise stated.

TABLE 3 Maternal antibiotic use and child asthma according to potential confounders in Manitoba, Canada, 1996–2012

	Dyads	Maternal antibiotic use during pregnancy	Childhood asthma	
			Cases	Incidence rate (95% CI) per 1000 person-years
All dyads	213 661	78 522 (36.8)	21 483	10.2 (10.0–10.3)
Maternal antibiotic use during pregnancy				
Yes	78 522	NA	9091	11.9 (11.6–12.1)
No	135 139	NA	12 392	9.2 (9.0–9.4)
Infant antibiotic use before age 12 months				
Yes	96 525	42 188 (43.7)	11 774	11.7 (11.4–11.9)
No	117 136	36 334 (31.0)	9709	8.8 (8.6–9.0)
Infant sex				
Male	109 437	40 267 (36.8)	12 615	11.8 (11.6–12.0)
Female	104 224	38 255 (36.7)	8868	8.5 (8.3–8.6)
Residence location#				
Urban	115 604	40 246 (34.8)	13 984	12.4 (12.2–12.6)
Rural	97 549	38 056 (39.0)	7458	7.6 (7.4–7.7)
Gestational age weeks				
<35	5016	1962 (39.1)	761	16.3 (15.2–17.5)
35–<37	9192	3594 (39.1)	1136	13.2 (12.4–14.0)
37–<39	43 799	16 429 (37.5)	4466	10.6 (10.3–11.0)
⩾39	155 654	56 537 (36.3)	15 120	9.7 (9.5–9.8)
Children in household#				
1	80 939	28 053 (34.7)	9273	11.7 (11.5–11.9)
2	71 403	26 138 (36.6)	7363	10.3 (10.1–10.6)
3	34 234	13 515 (39.5)	3040	8.9 (8.5–9.2)
⩾4	26 842	10 698 (39.9)	1777	6.7 (6.4–7.1)
Maternal asthma				
Yes	12 780	7180 (56.2)	2264	21.0 (20.1–21.9)
No	200 881	71 342 (35.5)	19 219	9.6 (9.4–9.7)
Neighbourhood income quintile#				
Q1 (lowest)	56 019	23 426 (41.8)	5832	10.6 (10.4–10.9)
Q2	44 214	16 637 (37.6)	4340	9.8 (9.5–10.1)
Q3	39 797	14 499 (36.4)	4000	10.1 (9.8–10.4)
Q4	39 017	12 962 (33.2)	3835	10.0 (9.6–10.2)
Q5 (highest)	34 106	10 778 (31.6)	3435	10.1 (9.8–10.5)
Maternal age years				
<20	20 308	9083 (44.7)	2010	9.8 (9.4–10.2)
20–24	46 589	19 700 (42.3)	4557	9.8 (9.5–10.1)
25–29	63 685	22 758 (35.7)	6359	10.1 (9.8–10.3)
30–34	55 474	18 160 (32.7)	5751	10.5 (10.3–10.8)
⩾35	27 605	8821 (32.0)	2806	10.5 (10.1–10.9)
Delivery method				
Caesarean section	41 248	15 557 (37.7)	4614	11.8 (11.5–12.2)
Vaginal	172 413	62 965 (36.5)	16 869	9.8 (9.6–9.9)
Birth weight g				
<3000	36 833	13 640 (37.0)	4322	12.0 (11.6–12.4)
3000–<3500	70 519	23 353 (36.0)	7013	10.1 (9.8–10.3)
3500–<4500	99 149	36 626 (36.9)	9492	9.6 (9.4–9.8)
⩾4500	6571	2613 (39.8)	656	10.1 (9.3–10.9)
Feeding method at birth				
Exclusively breastfed	108 147	36 856 (34.1)	11 127	9.9 (9.7–10.1)
Partially breastfed	60 891	22 686 (37.3)	5697	10.5 (10.2–10.8)
Formula-fed	41 026	17 470 (42.6)	4288	10.5 (10.1–10.8)

Data are presented as n or n (%), unless otherwise stated. n=213 661. NA: not applicable. #: excluding dyads with missing data, as reported in table 1.

following adjustment for sex, location of residence, gestational age, number of children in the household and maternal asthma (aHR 1.27, 95% CI 1.24–1.31), and was unchanged by further adjustment for postnatal antibiotic exposure (aHR 1.23, 95% CI 1.20–1.27) (table 4). There was no evidence of effect modification by infant sex, mode of delivery or infant feeding method (results not shown; p for interactions >0.40).

An apparent dose response was observed, demonstrating progressively increasing asthma risk with each additional course of maternal antibiotics during pregnancy: aHR 1.15 (95% CI 1.11–1.18) for one exposure, aHR 1.26 (95% CI 1.21–1.33) for two exposures and aHR 1.51 (95% CI 1.44–1.59) for three or more exposures (table 4, figure 1). When classified by type, most antibiotics were similarly associated with child asthma (table 4, figure 1), including β-lactam penicillins (aHR 1.22, 95% CI 1.18–1.25); macrolides, lincosamides and streptogramins (aHR 1.21, 95% CI 1.15–1.27); and sulphonamides and trimethoprim (aHR 1.28, 95% CI 1.19–1.37). However, other β-lactams (aHR 0.99, 95% CI 0.94–1.05) and tetracyclines, aminoglycosides and quinolones (aHR 1.06, 95% CI 1.01–1.12) were not significantly associated with child asthma.

The timing of maternal exposure did not modify the association of maternal antibiotic use and child asthma. Associations were similar for maternal antibiotic use during the first trimester (aHR 1.18, 95% CI 1.14–1.23), second trimester (aHR 1.15, 95% CI 1.11–1.19) and third trimester of pregnancy (aHR 1.18, 95% CI 1.13–1.22) (table 4). They were also similar for maternal antibiotic use during the 9 months before and after pregnancy (aHR 1.27, 95% CI 1.24–1.31 and aHR 1.32, 95% CI 1.28–1.36, respectively) (table 4, figure 1). Maternal antibiotic use before, during and after pregnancy was inter-correlated (online supplementary table S1); however sensitivity analyses excluding mothers who took antibiotics during more than one exposure period yielded similar results (table 5).

Discussion

In this population-based study, prenatal antibiotic exposure was associated with a 23% increased risk of asthma, independent of postnatal antibiotic exposure and several established asthma risk factors. There was an apparent dose response with repeated prenatal exposures; however, similar associations were observed for maternal antibiotic use before and after pregnancy. These results neither firmly support nor refute a directly causal pregnancy-specific relationship between maternal antibiotic use and childhood asthma; however, they contribute to the growing body of evidence linking early-life antibiotic exposure and asthma risk, and raise important questions for further research.

Our results are consistent with a case–control study by Metsälä *et al.* [26] showing that both prenatal and postnatal exposure to antibiotics were associated with an increased risk of asthma in Finnish children. In another case–control study, Mulder *et al.* [27] found that prenatal antibiotic exposure in the third

TABLE 4 Crude and adjusted estimates of the association between maternal antibiotic use and child asthma in Manitoba, Canada, 1996–2012

	Crude HR for child asthma	Adjusted HR for covariates[#]	Adjusted HR for covariates[#] + infant antibiotics[¶]
Dyads n	213661	213418	213418
Any maternal antibiotic use during pregnancy			
No	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Yes	1.29 (1.26–1.33)	1.27 (1.24–1.31)	1.23 (1.20–1.27)
Number of maternal antibiotic courses during pregnancy			
0	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
1	1.19 (1.15–1.23)	1.17 (1.14–1.21)	1.15 (1.11–1.18)
2	1.34 (1.28–1.40)	1.32 (1.26–1.38)	1.26 (1.21–1.32)
⩾3	1.60 (1.53–1.68)	1.59 (1.52–1.67)	1.51 (1.44–1.59)
Type of maternal antibiotic used during pregnancy			
β-lactams, penicillins	1.25 (1.21–1.28)	1.25 (1.22–1.29)	1.22 (1.18–1.25)
β-lactams, others	0.95 (0.89–1.00)	1.01 (0.95–1.07)	0.99 (0.94–1.05)
Macrolides, streptogramins, lincosamides	1.35 (1.29–1.42)	1.24 (1.18–1.30)	1.21 (1.15–1.27)
Tetracyclines, aminoglycosides, quinolones	1.11 (1.06–1.17)	1.07 (1.02–1.13)	1.06 (1.01–1.12)
Sulphonamides and trimethoprim	1.30 (1.21–1.39)	1.31 (1.22–1.40)	1.28 (1.19–1.37)
Timing of maternal antibiotic use			
9 months before pregnancy	1.39 (1.35–1.43)	1.31 (1.28–1.35)	1.27 (1.24–1.31)
Pregnancy trimester 1	1.26 (1.22–1.30)	1.21 (1.17–1.25)	1.18 (1.14–1.23)
Pregnancy trimester 2	1.20 (1.16–1.24)	1.18 (1.14–1.22)	1.15 (1.11–1.19)
Pregnancy trimester 3	1.14 (1.10–1.19)	1.20 (1.16–1.25)	1.18 (1.13–1.22)
9 months after pregnancy	1.42 (1.38–1.46)	1.37 (1.34–1.41)	1.32 (1.28–1.36)

Data are presented as hazard ratio (HR) (95% CI), unless otherwise stated. n=213661. [#]: covariates: maternal asthma, sex, location of residence, length of gestation, number of children in household and income quintile; [¶]: any infant antibiotic use before 12 months of age.

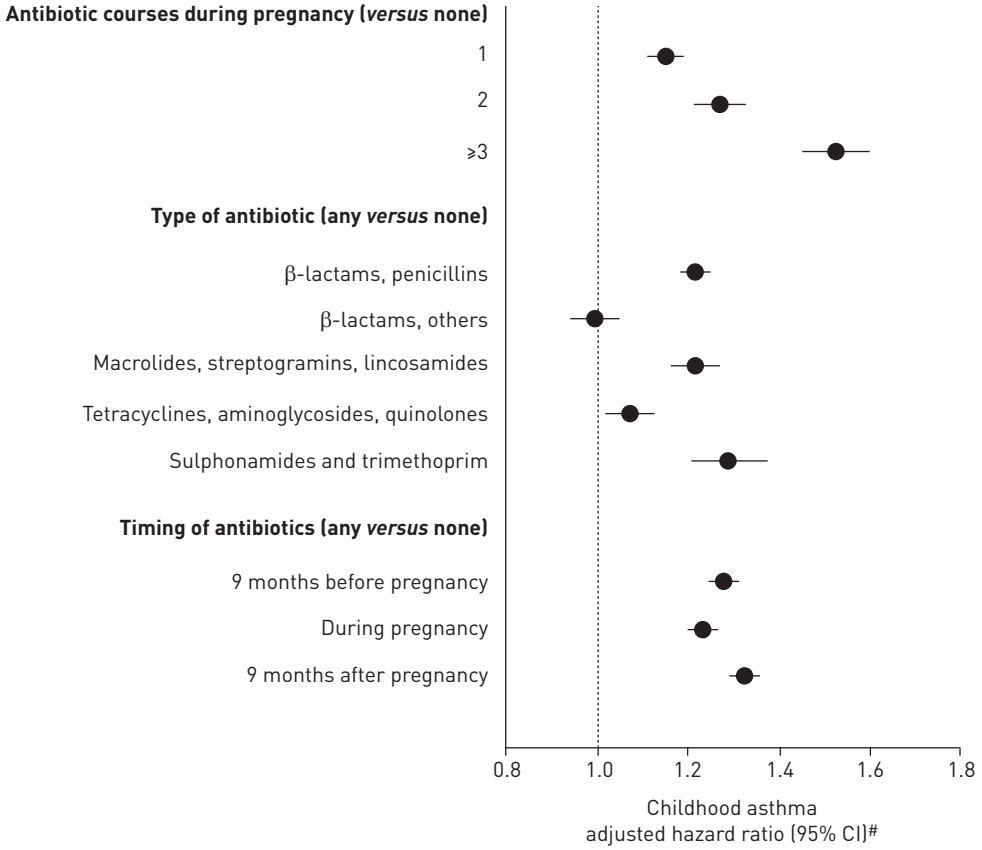

FIGURE 1 Associations between maternal antibiotics and childhood asthma by frequency, type and timing of antibiotic exposure among 213 418 mother–infant dyads in Manitoba, Canada, 1996-2012. #: adjusted for maternal asthma, sex, location of residence, length of gestation, number of children in household and postnatal antibiotic exposure in the first year of life, as described in table 4.

trimester of pregnancy was associated with an increased risk of asthma in Dutch children, with consistent results in a case–sibling sensitivity analysis. While these studies seem to support a causal relationship between prenatal antibiotic exposure and asthma development, other studies have used different approaches and challenged this hypothesis. For example, Örtqvist *et al.* [29] found that associations observed in Swedish children disappeared when using sibling controls, suggesting confounding by familial factors. In addition, Stokholm *et al.* [30] reported that maternal antibiotic use anytime from 80 weeks

TABLE 5 Sensitivity analysis for the association between maternal antibiotic use and child asthma in Manitoba, Canada, 1996–2012

	Main analysis Reference: no antibiotic use during exposure period of interest		Sensitivity analysis# Reference: no antibitoic use from 9 months before until 9 months after pregnancy	
	Dyads	aHR¶ (95% CI) for child asthma	Dyads	aHR¶ (95% CI) for child asthma
9 months before pregnancy	213 418	1.31 (1.28–1.35)	115 891	1.25 (1.21–1.30)
During pregnancy	213 418	1.27 (1.24–1.31)	114 283	1.21 (1.16–1.25)
9 months after pregnancy	213 418	1.37 (1.34–1.41)	115 975	1.32 (1.28–1.37)

Data are presented as n, unless otherwise stated. aHR: adjusted hazard ratio. #: to isolate the effect of antibiotic use during each of the three exposure periods of interest, mothers were excluded if they used antibiotics during more than one exposure period; ¶: models are adjusted for maternal asthma, sex, location of residence, length of gestation, number of children in household and income quintile.

prior to 80 weeks following pregnancy was similarly associated with childhood asthma in Denmark. The authors speculated that maternal propensity for infection (rather than antibiotic use) is the causal factor linking prenatal antibiotic exposure with asthma development. Örtqvist *et al.* [36] recently confirmed this phenomenon in Sweden, finding similar associations for maternal exposure before, during and after pregnancy. Consistent with Stokholm *et al.* [30] and Örtqvist *et al.* [36], we have found that maternal antibiotic use before, during and after pregnancy is similarly associated with increased asthma risk in offspring. These results suggest the link between maternal antibiotic use and asthma in offspring is either not directly causal or not specific to pregnancy.

There are several potential explanations for a non-causal association between maternal antibiotic use and childhood asthma. First, the observed association may be confounded by healthcare utilisation patterns or other unmeasured factors that are shared within families, such as smoking and environmental exposures. Second, as Stokholm *et al.* [30] proposed, it is possible that maternal antibiotic use is a marker of genetic susceptibility to infections, which is inherited by offspring and imparts a predisposition for asthma. Third, as suggested by Weiss and Litonjua [38], a maternal deficiency in vitamin D or other immunomodulatory nutrient could explain both the increased risk of infection in mothers and increased risk of asthma in offspring. Finally, since maternal and child medication usage are strongly associated [39], it is possible that maternal antibiotic use is a surrogate for infant antibiotic use, which is known to influence asthma development [17, 18]. While we could not address environmental exposures, genetics or nutritional hypotheses in our administrative database study, our findings do not support the final explanation, since our results were unchanged following adjustment for infant antibiotic use.

Another approach to address confounding in prenatal exposure studies is to evaluate fathers' exposures as a negative control. Mulder *et al.* [27] reported that maternal (but not paternal) antibiotic use during pregnancy was associated with child asthma at the age of 5 years, supporting a causal effect from *in utero* exposure. In contrast, Örtqvist *et al.* [36] showed that both maternal and paternal antibiotic use during pregnancy were associated with child asthma before 2.5 years, suggesting confounding by shared familial factors. Notably, however, the maternal association was stronger and persistent throughout childhood, whereas paternal exposure was not associated with child asthma after 2.5 years. Thus, while we could not address paternal exposure in our study, this approach warrants further investigation.

Notably, the dose response observed by Stokholm *et al.* [30] and others [26, 28, 31], and replicated in our study, suggests that antibiotics or some related underlying factor (whether genetic, nutritional or environmental) may be causally related to asthma development in offspring. While this pattern could indicate dose response in a confounder, research is warranted to pursue these hypotheses in other settings where causal mechanisms can be explored and tested, such as clinical cohorts and animal models. It must also be acknowledged that, while the overuse of antibiotics can promote antimicrobial resistance and microbiome dysbiosis, untreated infections can also be harmful, especially to a developing fetus. For example, urinary tract infections during pregnancy are associated with intrauterine growth restriction, preterm labour and miscarriage [40, 41]. Keeping this risk–benefit balance in mind, it is important to study and clarify the potentially unintended consequences of prenatal antibiotic exposure.

It is conceivable that maternal antibiotic use before, during and after pregnancy could impact infant microbiota and subsequent immune development. Pre-gestational antibiotic use may have long-term effects on the maternal microbiota that persist during pregnancy, and postpartum antibiotics could influence the transmission of maternal skin and breast milk microbiota to the infant [37]. Consistent with Mulder *et al.* [27], our finding that different types of antibiotics are differentially associated with child asthma lends support to this hypothesis, since different antibiotics will differentially impact maternal microbiota and their transmission to the infant. However, exposures occurring closer to the time of this microbial transfer would be expected to have a greater impact. The absence of any temporal gradient in our results, and those of Stokholm *et al.* [30], points to the involvement of additional mechanisms, as discussed later. Future research is needed to determine whether antibiotic-induced disruption of the maternal microbiota before pregnancy may persist during pregnancy or postpartum, whether postnatal maternal antibiotics impact maternal–infant sharing of microbes after pregnancy and whether these potential effects may influence asthma development.

Strengths of this study include the large unselected population, capturing virtually all children born in the province of Manitoba over an 18-year period, and the use of administrative healthcare data to objectively document asthma diagnoses, hospitalisations and antibiotic exposures. Using healthcare records eliminates recall bias, minimises loss to follow-up and provides key details that are not accurately captured by self-report, including the specific antibiotic type, dose and timing of exposure. Unlike most previous studies, we mutually adjusted our analyses for prenatal and postnatal antibiotic exposure during the first year of life; an important adjustment since maternal and infant healthcare utilisation tend to be correlated [39], but

could be independently associated with child asthma development. In addition, we performed sensitivity analyses for maternal antibiotic use before and after pregnancy, although sibling controls and paternal exposures were not examined. Finally, our results confirm previous research [19] identifying maternal asthma, male sex, urban residence, premature birth, lower birth order, and antibiotic use during infancy as significant risk factors for asthma.

A limitation of our study is the lack of information about the indication for antibiotic treatment. In addition, exposure misclassification is possible since we cannot confirm patient compliance with filled prescriptions, and our database does not capture antibiotics administered in hospital. Thus, we could not account for intrapartum antibiotic prophylaxis for group B *Streptococcus*, which affects >20% of deliveries in Manitoba [42] and has been shown to influence infant gut microbiota development [43]. In addition, we could not account for indirect exposure to antibiotics in food or the environment [44]. Outcome misclassification is also possible, since asthma is commonly misdiagnosed in young children; however, we evaluated multiple disease definitions and required evidence of serious (hospitalisation) or persistent (multiple diagnoses or prescriptions) disease after 5 years of age to maximise specificity. Finally, confounding bias is possible since we could not account for potential confounders that are not captured in administrative databases, such as maternal and child nutrition, education, smoking and environmental exposures including pets, tobacco smoke, mould and daycare attendance.

Conclusions

In this province-wide study, we observed a dose-dependent association between maternal antibiotic use and asthma risk in offspring; however, this association was not specific to antibiotic use during pregnancy. Further research is needed to better understand the nature of this association and address intrapartum antibiotic exposure. While our current results do not firmly support nor refute a directly causal pregnancy-specific relationship between maternal antibiotic use and childhood asthma, it remains important to use antibiotics judiciously.

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