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Early View

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

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Short Title: Prenatal antibiotics and childhood asthma

Key Words: asthma, antibiotics, pregnancy, DOHaD, administrative database study

Take home message: Maternal antibiotic use is associated with childhood asthma, but the association is not specific to antibiotic use during pregnancy.

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 characterize the association between prenatal antibiotic exposure and childhood asthma.

We performed a population-based cohort study using prescription records, hospitalization records, and physician billing claims from 213,661 mother-child dyads born in Manitoba, Canada from 1996-2012. 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 prenatally exposed to antibiotics, and 10.1% developed asthma. Prenatal antibiotic exposure was associated with an increased risk of asthma (adjusted HR 1.23, 95%CI 1.20-1.27). There was an apparent dose-response (aHRs: 1.15, 1.11-1.18 for 1 course; 1.26, 1.21-1.32 for 2 courses; 1.51, 1.44-1.59 for ≥3 courses). Maternal antibiotic use during 9 months before pregnancy (1.27, 1.24-1.31) and 9 months postpartum (1.32, 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.

Introduction

Asthma is the most common chronic disease of childhood[1], affecting over 10% of children worldwide[2]. In the United States, the total cost of asthma to society is estimated at $56 billion per year[3]. Similarly, in Canada asthma is a leading cause of healthcare utilization, 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 Center for Disease Control reports that antibiotics are prescribed at 12.6% of all ambulatory care visits, and 30% of these prescriptions may be unnecessary[5]. Across different settings, between 20-40% of women are prescribed antibiotics during pregnancy[6–9], accounting for nearly 80% of all drugs used by pregnant women[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; 685,550 participants)[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 previous 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 of this association.

Using administrative health data capturing all children born in Manitoba, Canada from 1996-2012, 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. We also 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, hospitalization 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 anonymized 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 at least 1 year before and 1 year after pregnancy, and the child was continuously registered for at least 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 vs. 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 Anatomic Therapeutic Chemical (ATC) classification system as follows: B-lactam penicillins (J01C); other B-lactams (J01D); macrolides, streptogramins, lincosamides (J01F); sulphonamides and trimethoprim (J01E), and finally, 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 5 years of age: 1) any hospitalization for asthma; or 2) ≥2 physician diagnoses of asthma, at least 3 months apart and

within a 1 year period; or 3) ≥2 prescriptions for asthma medications within a 1 year period. The age requirement was applied because five years is the minimum age for confirming asthma diagnosis by lung function testing and misdiagnosis is common before this age[35]. We used the actual index date (the date when the child first met any of the above criteria, which could be prior to age 5) to capture incident cases for survival analysis. This definition was based on the validated definition applied by Kozyrskyj et al. using the same administrative database[17]. We modified this definition to increase specificity by requiring repeated physician diagnoses to be at least 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 above). 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 childhood asthma were estimated using Cox regression models and reported as crude and adjusted hazard ratios (HR, aHR) and 95% confidence intervals (CI), 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 modeled 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. Beta-lactam penicillins were the most commonly prescribed type of antibiotic (24.6%), with fewer mothers receiving other beta-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% vs. 35.5% among mothers with vs. without asthma, p<0.0001), and their children had a higher rate of asthma (21.0 vs. 9.6 cases per 1000 person-years, p <0.0001) **(Table 3)**. Preterm birth was also 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, 95%CI 9.0-9.4) **(Table 3)** (crude HR 1.29, 95%CI 1.26-1.33). This association persisted 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 1 exposure, aHR 1.26 (95%CI 1.21-1.33) for 2 exposures, and aHR 1.51 (95%CI 1.44-1.59) for 3 or more exposures **(Table 4, Figure 1)**. When classified by type, most antibiotics were similarly associated with child asthma **(Table 4, Figure 1)**, including beta-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 beta-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**). Sensitivity analyses excluding mothers who took antibiotics during more than one exposure period were again similar before, during, and after pregnancy (aHR 1.25, 95%CI 1.21-1.30, aHR 1.21, 95%CI 1.16-1.25, and aHR 1.32, 95%CI 1.28-1.37, respectively) **(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 do not 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 Metsala et al. showing that both prenatal and postnatal exposure to antibiotics were associated with an increased risk of asthma in Finnish children[26]. In another case-control study, Mulder et al. found that prenatal antibiotic exposure in the third trimester of pregnancy was associated with an increased risk of asthma in Dutch children, with consistent results in a case-sibling sensitivity analysis[27]. 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, Ortqvist et al. found that associations observed in Swedish children disappeared when using sibling controls, suggesting confounding by familial factors[29]. In addition, Stokholm et al[30] recently reported that maternal antibiotic use anytime from 80 weeks before 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. Ortqvist et al. recently confirmed this phenomenon in Sweden, finding similar associations for maternal exposure before, during and after pregnancy[36]. Consistent with Stokholm et al. and Ortqvist et al., 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.

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 post-partum 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., points to the involvement of additional mechanisms, as discussed below. Future research is needed to determine whether antibiotic-induced disruption of the maternal microbiota before pregnancy may persist until pregnancy or post-partum, whether the postnatal maternal antibiotics impact maternal-infant sharing of microbes after pregnancy, and whether these potential effects may influence asthma development.

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 utilization patterns or other unmeasured factors that are shared within families, such as smoking and environmental exposures. Second, as Stokholm et al. 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[30]. Third, as Weiss et al. suggested, a maternal deficiency in vitamin D or other immunomodulatory nutrient could explain the increased risk of infection in mothers and increased risk of asthma in offspring [38]. 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.

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 also 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 labor, 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.

Another approach to address confounding in prenatal exposure studies is to evaluate fathers' exposures as a negative control. Mulder et al. reported that maternal (but not paternal) antibiotic use during pregnancy was associated with child asthma at age 5, supporting a causal effect from *in utero* exposure[27]. In contrast, Ortqvist et al. showed that both maternal and paternal antibiotic use during pregnancy were associated with child asthma before 2.5 years[36], 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.

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, hospitalizations, and antibiotic exposures. Using healthcare records eliminates recall bias, minimizes 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 utilization tend to be correlated[39] but could be independently associated with child asthma development. We also 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, and lower birth order 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 over 20% of deliveries in Manitoba[42] and has been shown to influence infant gut microbiota development[43]. We also 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 (hospitalization) or persistent (multiple diagnoses or prescriptions) disease after 5 years of age to maximize 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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References

1. Asher I, Pearce N. Global burden of asthma among children. *Int J Tuberc Lung Dis*. 2014;18(11):1269-1278.

2. Pearce N, Aït-Khaled N, Beasley R, et al. Worldwide trends in the prevalence of asthma symptoms: phase III of the International Study of Asthma and Allergies in Childhood (ISAAC). *Thorax*. 2007;62(9):758-766. doi:10.1136/thx.2006.070169.

3. Bahadori K, Doyle-Waters MM, Marra C, et al. Economic burden of asthma: a systematic review. *BMC Pulm Med*. 2009;9:24. doi:10.1186/1471-2466-9-24.

4. Ismaila AS, Sayani AP, Marin M, Su Z. Clinical, economic, and humanistic burden of asthma in Canada: a systematic review. *BMC Pulm Med*. 2013;13:70. doi:10.1186/1471-2466-13-70.

5. Fleming-Dutra KE, Hersh AL, Shapiro DJ, et al. Prevalence of Inappropriate Antibiotic Prescriptions Among US Ambulatory Care Visits, 2010-2011. *JAMA*. 2016;315(17):1864. doi:10.1001/jama.2016.4151.

6. Brandon Bookstaver P, Bland CM, Griffin B, Stover KR, Eiland LS, McLaughlin M. A review of antibiotic use in pregnancy. *Pharmacotherapy*. 2015;35(11):1052-1062. doi:10.1002/phar.1649.

7. Broe A, Pottegård A, Lamont RF, Jørgensen JS, Damkier P. Increasing use of antibiotics in pregnancy during the period 2000-2010: Prevalence, timing, category, and demographics. *BJOG An Int J Obstet Gynaecol*. 2014;121(8):988-996. doi:10.1111/1471-0528.12806.

8. de Jonge L, Jens Bos H, van Langen IM, de Jong-van den Berg LTW, Bakker MK. Antibiotics prescribed before, during and after pregnancy in the Netherlands: a drug utiliztion study. *Pharmacoepidemiol Drug Saf*. 2014;23:60-68. doi:10.1002/pds.

9. Santos F, Oraichi D, Bérard A. Prevalence and predictors of anti-infective use during pregnancy. *Pharmacoepidemiol Drug Saf*. 2010;19:418-427. doi:10.1002/pds.

10. Nahum G, Uhl K, Kennedy D. Antibiotic use in pregnancy and lactation: what is and is not known about teratogenic and toxic risks. *Obstet Gynecol*. 2006;107(5):1120-1138. doi:10.1097/01.AOG.0000216197.26783.b5.

11. Arrieta M, Stiemsma LT, Dimitriu PA, et al. Early infancy microbial and metabolic alterations affect risk of childhood asthma. *Sci Transl Med*. 2015;7(307).

12. Kuo CH, Kuo HF, Huang CH, Yang SN, Lee MS, Hung CH. Early life exposure to antibiotics and the risk of childhood allergic diseases: An update from the perspective of the hygiene hypothesis. *J Microbiol Immunol Infect*. 2013;46(5):320-329. doi:10.1016/j.jmii.2013.04.005.

13. Stokholm J, Schjørring S, Eskildsen CE, et al. Antibiotic use during pregnancy alters the commensal vaginal microbiota. *Clin Microbiol Infect*. 2014;20(7):629-635. doi:10.1111/1469-0691.12411.

14. Grönlund M, Gueimonde M, Laitinen K, et al. Maternal breast-milk and intestinal

bifidobacteria guide the compositional development of the bifidobacterium microbiota in infants at risk of allergic disease. *Pediatriya*. 2012;52(3):57-63. doi:10.1111/j.1365-2222.2007.02849.x.

15. Keski-Nisula L, Kyynarainen HR, Kyynarainen U, Karhukorpi J, Heinonen S, Pekkanen J. Maternal intrapartum antibiotics and decreased vertical transmission of Lactobacillus to neonates during birth. *Acta Paediatr Int J Paediatr*. 2013;102(5):480-485. doi:10.1111/apa.12186.

16. Hoskin-Parr L, Teyhan A, Blocker A, Henderson AJW. Antibiotic exposure in the first two years of life and development of asthma and other allergic diseases by 7.5 yr: a dose-dependent relationship. *Pediatr Allergy Immunol*. 2013;24(8):762-771. doi:10.1111/pai.12153.

17. Kozyrskyj AL, Ernst PE, Becker AB. Increased Risk of Childhood Asthma From Antibiotic Use in Early Life. *Chest*. 2007;131:1753-1759. doi:10.1378/chest.06-3008.

18. Risnes KR, Belanger K, Murk W, Bracken MB. Antibiotic Exposure by 6 Months and Asthma and Allergy at 6 Years: Findings in a Cohort of 1,401 US Children. *Am J Epidemiol*. 2011;173(3):310-318. doi:10.1093/aje/kwq400.

19. Castro-Rodriguez JA, Forno E, Rodriguez-Martinez CE, Celedón JC. Risk and Protective Factors for Childhood Asthma: What Is the Evidence? *J Allergy Clin Immunol Pract*. 2016;(3). doi:10.1016/j.jaip.2016.05.003.

20. Murk W, Risnes KR, Bracken MB. Prenatal or early-life exposure to antibiotics and risk of childhood asthma: a systematic review. *Pediatrics*. 2011;127(6):1125-1138. doi:10.1542/peds.2010-2092.

21. Penders J, Kummeling I, Thijs C. Infant antibiotic use and wheeze and asthma risk: A systematic review and meta-analysis. *Eur Respir J*. 2011;38(2):295-302. doi:10.1183/09031936.00105010.

22. Almqvist C, Wettermark B, Hedlin G, Ye W, Lundholm C. Antibiotics and asthma medication in a large register-based cohort study - confounding, cause and effect. *Clin Exp Allergy*. 2012;42(1):104-111. doi:10.1111/j.1365-2222.2011.03850.x.

23. Lamont HF, Blogg HJ, Lamont RF. Safety of antimicrobial treatment during pregnancy: a current review of resistance, immunomodulation and teratogenicity. *Expert Opin Drug Saf*. 2014;13(12):1569-1581. doi:10.1517/14740338.2014.939580.

24. Collier CH, Risnes K, Norwitz ER, Bracken MB, Illuzzi JL. Maternal infection in pregnancy and risk of asthma in offspring. *Matern Child Health J*. 2013;17(10):1940-1950. doi:10.1007/s10995-013-1220-2.

25. Lapin B, Piorkowski J, Ownby D, et al. Relationship between prenatal antibiotic use and asthma in at-risk children. *Ann Allergy, Asthma Immunol*. 2015;114(3):203-207. doi:10.1016/j.anai.2014.11.014.

26. Metsälä J, Lundqvist A, Virta LJ, Kaila M, Gissler M, Virtanen SM. Prenatal and post-natal exposure to antibiotics and risk of asthma in childhood. *Clin Exp Allergy*.

2015;45(1):137-145. doi:10.1111/cea.12356.

27. Mulder B, Pouwels KB, Schuiling-Veninga CCM, et al. Antibiotic use during pregnancy and asthma in preschool children: the influence of confounding. *Clin Exp Allergy*. 2016;7576. doi:10.1111/cea.12756.

28. Stensballe LG, Simonsen J, Jensen SM, Bønnelykke K, Bisgaard H. Use of Antibiotics during Pregnancy Increases the Risk of Asthma in Early Childhood. *J Pediatr*. 2013;162(4):832-838.e3. doi:10.1016/j.jpeds.2012.09.049.

29. Örtqvist AK, Lundholm C, Kieler H, et al. Antibiotics in fetal and early life and subsequent childhood asthma: nationwide population based study with sibling analysis. *BMJ*. 2014;349(November):g6979. doi:10.1136/bmj.g6979.

30. Stokholm J, Sevelsted A, Bønnelykke K, Bisgaard H. Maternal propensity for infections and risk of childhood asthma: a registry-based cohort study. *Lancet Respir Med*. 2014;2(8):631-637. doi:10.1016/S2213-2600(14)70152-3.

31. McKeever TM, Lewis SA, Smith C, Hubbard R. The importance of prenatal exposures on the development of allergic disease: A birth cohort study using the West Midlands General Practice Database. *Am J Respir Crit Care Med*. 2002;166(6):827-832. doi:10.1164/rccm.200202-158OC.

32. Roos LL, Nicol PJ. A research registry: Uses, development, and accuracy. *J Clin Epidemiol*. 1999;52(1):39-47. doi:10.1016/S0895-4356(98)00126-7.

33. Kozyrskyj A, Mustard C. Validation of an electronic, population-based prescription database. *Ann Pharmacother*. 1998;32:1152-1157.

34. Robinson J, Young T, Roos L, Gelskey D. Estimating the burden of disease: comparing administrative data and self reports. *Med Care*. 1997;35:932-947.

35. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention (2015 update). 2015:148. doi:10.1183/09031936.00138707.

36. Örtqvist AK, Lundholm C, Fang F, Fall T, Almqvist C. Parental antibiotics and childhood asthma—a population-based study. *J Allergy Clin Immunol Pract*. 2017;5(5):1451-1454.e4. doi:10.1016/j.jaip.2017.03.009.

37. Blaser MJ, Bello MGD. Maternal antibiotic use and risk of asthma in offspring. *Lancet Respir Med*. 2014;2(10):e16. doi:10.1016/S2213-2600(14)70219-X.

38. Weiss ST, Litonjua AA. Maternal antibiotic use and childhood asthma: The missing link? *Lancet Respir Med*. 2014;2(8):597-598. doi:10.1016/S2213-2600(14)70122-5.

39. Janicke D, Finney J, Riley a. Children's health care use: A prospective investigation of factors related to care-seeking. *Med Care*. 2001;39(9):990-1001.

40. Vazquez JC, Abalos E. Treatments for symptomatic urinary tract infections during pregnancy. *Cochrane Database Syst Rev*. 2011;(1). doi:10.1002/14651858.CD002256.pub2.www.cochranelibrary.com.

41. Harbison AF, Polly DM, Musselman ME. Antiinfective Therapy for Pregnant or Lactating Patients in the Emergency Department. *Am J Heal Pharm*. 2015;72. doi:10.2146/ajhp130797.

42. Persaud RR, Azad MB, Chari RS, et al. Perinatal antibiotic exposure of neonates in Canada and associated risk factors : a population- based study Perinatal antibiotic exposure of neonates in Canada and associated risk factors : a population-based study. *J Matern Neonatal Med*. 2015;28(10):1190-1195. doi:10.3109/14767058.2014.947578.

43. Azad MB, Konya T, Persaud RR, et al. Impact of maternal intrapartum antibiotics , method of birth and breastfeeding on gut microbiota during the first year of life : a prospective cohort study. *BJOG*. 2016;123:983-993. doi:10.1111/1471-0528.13601.

44. Francino MP. Antibiotics and the human gut microbiome: Dysbioses and accumulation of resistances. *Front Microbiol*. 2016;6(JAN):1-11. doi:10.3389/fmicb.2015.01543.

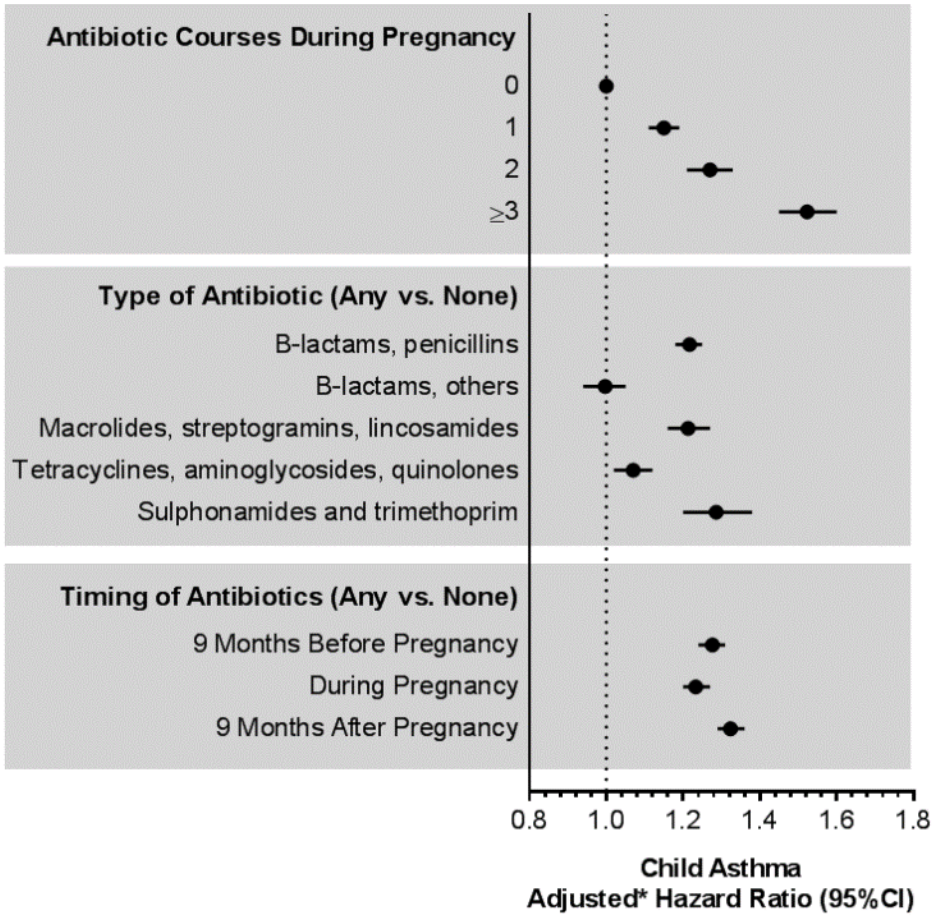

Figure 1. Associations between maternal antibiotics and childhood asthma by frequency, type and timing of antibiotic exposure. *Adjusted for maternal asthma, sex, location of residence, length of gestation, number of children in household, and postnatal antibiotic exposure in the first year of life.

Table 1. Characteristics of study population: mother-infant dyads in Manitoba, Canada, 1996-2012 (N=213,661).

	Mean	± SD
Maternal Age (years)	27.6	± 5.9
Infant Birth Weight (grams)	3493	± 619
Gestational Age (weeks)	39.1	± 1.9
	N	%
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	3,597	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%

Table 2. Maternal and infant antibiotic use in Manitoba, Canada, 1996-2012 (N=213,661)

	n	%
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 or more	13,282	6.2%
Type of maternal antibiotics used during pregnancy		
B-lactams, penicillins	52,598	24.6%
B-lactams, others	12,998	6.1%
Macrolides, streptogramins, lincosamides	15,120	7.1%
Tetracyclines, quinolones, others	16,074	7.5%
Sulphonamides and trimethoprim	5,615	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%

Table 3. Maternal antibiotic use and child asthma according to potential confounders in Manitoba, Canada, 1996-2012 (N=213,661).

Characteristic		Maternal Antibiotic Use During Pregnancy		Childhood Asthma	
	N	n	%	n	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	n/a	n/a	9,091	11.9 (11.6 - 12.1)
No	135,139	n/a	n/a	12,392	9.2 (9.0 - 9.4)
Infant antibiotic use before 12 months of age					
Yes	96,525	42,188	43.7%	11,774	11.7 (11.4 - 11.9)
No	117,136	36,334	31.0%	9,709	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%	8,868	8.5 (8.3 - 8.6)
Residence location[a]					
Urban	115,604	40,246	34.8%	13,984	12.4 (12.2 - 12.6)
Rural	97,549	38,056	39.0%	7,458	7.6 (7.4 - 7.7)
Gestational age (weeks)					
<35	5,016	1,962	39.1%	761	16.3 (15.2 - 17.5)
35 to <37	9,192	3,594	39.1%	1,136	13.2 (12.4 - 14.0)
37 to <39	43,799	16,429	37.5%	4,466	10.6 (10.3 - 11.0)
39+	155,654	56,537	36.3%	15,120	9.7 (9.5 - 9.8)
Children in household[a]					
1	80,939	28,053	34.7%	9,273	11.7 (11.5 - 11.9)
2	71,403	26,138	36.6%	7,363	10.3 (10.1 - 10.6)
3	34,234	13,515	39.5%	3,040	8.9 (8.5 - 9.2)
4+	26,842	10,698	39.9%	1,777	6.7 (6.4 - 7.1)
Maternal asthma					
Yes	12,780	7,180	56.2%	2,264	21.0 (20.1 - 21.9)
No	200,881	71,342	35.5%	19,219	9.6 (9.4 - 9.7)
Neighbourhood income quintile[a]					
Q1 (lowest)	56,019	23,426	41.8%	5,832	10.6 (10.4 - 10.9)
Q2	44,214	16,637	37.6%	4,340	9.8 (9.5 - 10.1)
Q3	39,797	14,499	36.4%	4,000	10.1 (9.8 - 10.4)
Q4	39,017	12,962	33.2%	3,835	10.0 (9.6 - 10.2)
Q5 (highest)	34,106	10,778	31.6%	3,435	10.1 (9.8 - 10.5)
Maternal age (years)					
<20	20,308	9,083	44.7%	2,010	9.8 (9.4 - 10.2)
20-24	46,589	19,700	42.3%	4,557	9.8 (9.5 - 10.1)
25-29	63,685	22,758	35.7%	6,359	10.1 (9.8 - 10.3)
30-34	55,474	18,160	32.7%	5,751	10.5 (10.3 - 10.8)
35+	27,605	8,821	32.0%	2,806	10.5 (10.1 - 10.9)
Delivery method					
Caesarean section	41,248	15,557	37.7%	4,614	11.8 (11.5 - 12.2)
Vaginal	172,413	62,965	36.5%	16,869	9.8 (9.6 - 9.9)
Birth weight (grams)					
<3000	36,833	13,640	37.0%	4,322	12.0 (11.6 - 12.4)
3000 to <3500	70,519	23,353	36.0%	7,013	10.1 (9.8 - 10.3)
3500 to <4500	99,149	36,626	36.9%	9,492	9.6 (9.4 - 9.8)
4500+	6,571	2,613	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%	5,697	10.5 (10.2 – 10.8)
Formula Fed	41,026	17,470	42.6%	4,288	10.5 (10.1 – 10.8)

CI, confidence interval. [a]Excluding dyads with missing data, as reported in Table 1.

Table 4. Crude and adjusted estimates of the association between maternal antibiotic use and child asthma in Manitoba, Canada, 1996-2012 (N=213,661).

Antibiotic use	Crude HR for child asthma	Adjusted for Covariates[a]	Adjusted for Covariates[a] + Infant Antibiotics[b]
	N=213,661	N=213,418	N=213,418
	HR (95% CI)	HR (95% CI)	HR (95% CI)
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			
B-lactams, penicillins	1.25 (1.21 - 1.28)	1.25 (1.22 - 1.29)	1.22 (1.18 - 1.25)
B-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)

HR, hazard ratio; CI, confidence interval. [a]Covariates: maternal asthma, sex, location of residence, length of gestation, number of children in household, and income quintile. [b]Any infant antibiotic use before 12 months of age.

Table 5. Sensitivity analysis for the association between maternal antibiotic use and child asthma in Manitoba, Canada, 1996-2012.

Exposure Period for Maternal Antibiotic Use	Main Analysis Reference: no antibiotic use during exposure period of interest		Sensitivity Analysis[b] Reference: no antibitoic use from 9 months before until 9 months after pregnancy	
	N	Adjusted[a] HR (95%CI) for child asthma	N	Adjusted[a] HR (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)

[a]Models are adjusted for maternal asthma, sex, location of residence, length of gestation, number of children in household, and income quintile. [b]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.

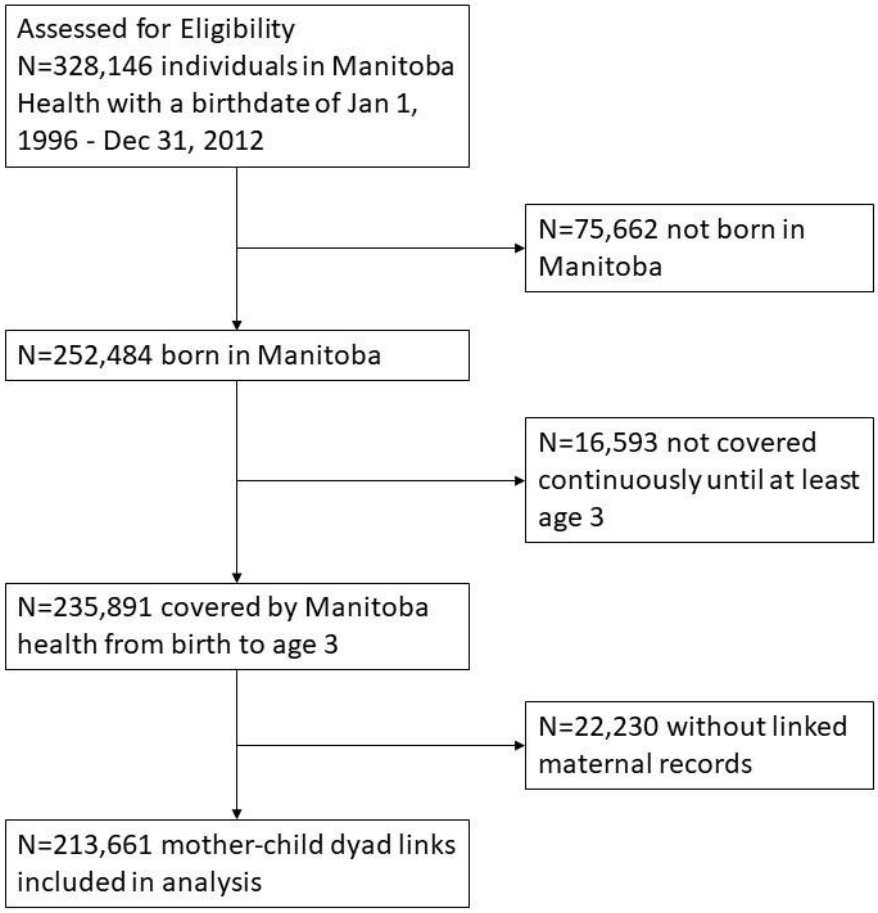

Figure S1. CONSORT Flow Diagram of individuals included in study

Table S1. Correlation of maternal antibiotic use before, during and after pregnancy.

Exposure Period	Maternal Antibiotic Use	N	% Maternal Antibiotic Use by Exposure Period				
			9 months before Pregnancy	Pregnancy Trimester 1	Pregnancy Trimester 2	Pregnancy Trimester 3	9 months after Pregnancy
Overall		216,661	35.2%	16.2%	18.4%	14.7%	35.5%
9 months before Pregnancy	No	138,495	0.0%	11.4%	13.9%	11.5%	28.4%
	Yes	75,166		24.9%	26.7%	20.4%	48.5%
Pregnancy Trimester 1	No	179,099	31.5%	0.0%	15.6%	12.7%	32.1%
	Yes	34,562	54.1%		33.0%	25.0%	53.1%
Pregnancy Trimester 2	No	174,321	31.6%	13.3%	0.0%	11.7%	31.7%
	Yes	39,340	51.0%	29.0%		27.8%	52.1%
Pregnancy Trimester 3	No	182,331	32.8%	14.2%	15.6%	0.0%	32.6%
	Yes	31,330	49.0%	27.6%	34.9%		51.8%
9 months after Pregnancy	No	137,900	28.0%	11.8%	13.7%	10.9%	0.0%
	Yes	75,761	48.2%	24.2%	27.1%	21.4%	

Shading is proportional to the proportion of maternal antibiotic use (0% = white, 100% = black).