Decreasing antibiotic use, the gut microbiota, and asthma incidence in children: evidence from population-based and prospective cohort studies

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Summary

Background Childhood asthma incidence is decreasing in some parts of Europe and North America. Antibiotic use in infancy has been associated with increased asthma risk. In the present study, we tested the hypothesis that decreases in asthma incidence are linked to reduced antibiotic prescribing and mediated by changes in the gut bacterial community.

Methods This study comprised population-based and prospective cohort analyses. At the population level, we used administrative data from British Columbia, Canada (population 4·7 million), on annual rates of antibiotic prescriptions and asthma diagnoses, to assess the association between antibiotic prescribing (at age <1 year) and asthma incidence (at age 1–4 years). At the individual level, 2644 children from the Canadian Healthy Infant Longitudinal Development (CHILD) prospective birth cohort were examined for the association of systemic antibiotic use (at age <1 year) with the diagnosis of asthma (at age 5 years). In the same cohort, we did a mechanistic investigation of 917 children with available 16S rRNA gene sequencing data from faecal samples (at age ≤1 year), to assess how composition of the gut microbiota relates to antibiotic exposure and asthma incidence.

Findings At the population level between 2000 and 2014, asthma incidence in children (aged 1–4 years) showed an absolute decrease of 7·1 new diagnoses per 1000 children, from 27·3 (26·8–28·3) per 1000 children to 20·2 (19·5–20·8) per 1000 children (a relative decrease of 26·0%). Reduction in incidence over the study period was associated with decreasing antibiotic use in infancy (age <1 year), from 1253·8 prescriptions (95% CI 1219·3–1288·9) per 1000 infants to 489·1 (467·6–511·2) per 1000 infants (Spearman's r=0·81; p<0·0001). Asthma incidence increased by 24% with each 10% increase in antibiotic prescribing (adjusted incidence rate ratio 1·24 [95% CI 1·20–1·28]; p<0·0001). In the CHILD cohort, after excluding children who received antibiotics for respiratory symptoms, asthma diagnosis in childhood was associated with infant antibiotic use (adjusted odds ratio [aOR] 2·15 [95% CI 1·37–3·39]; p=0·0009), with a significant dose–response; 114 (5·2%) of 2182 children unexposed to antibiotics had asthma by age 5 years, compared with 23 (8·1%) of 284 exposed to one course, five (10·2%) of 49 exposed to two courses, and six (17·6%) of 34 exposed to three or more courses (aOR 1·44 [1·16–1·79]; p=0·0008). Increasing α-diversity of the gut microbiota, defined as an IQR increase (25th to 75th percentile) in the Chao1 index, at age 1 year was associated with a 32% reduced risk of asthma at age 5 years (aOR for IQR increase 0·68 [0·46–0·99]; p=0·046). In a structural equation model, we found the gut microbiota at age 1 year, characterised by α-diversity, β-diversity, and amplicon sequence variants modified by antibiotic exposure, to be a significant mediator between outpatient antibiotic exposure in the first year of life and asthma diagnosis at age 5 years (β=0·08; p=0·027).

Interpretation Our findings suggest that the reduction in the incidence of paediatric asthma observed in recent years might be an unexpected benefit of prudent antibiotic use during infancy, acting via preservation of the gut microbial community.

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Introduction

Asthma is one of the most common chronic diseases in children,[1] with a prevalence of at least 8% reported among children in Canada and the USA between 2014 and 2018. This increase in prevalence has occurred in recent decades; in the UK and the USA, prevalence doubled between 1955 and 2000, from around 4% to 8–10%, and then plateaued between 2000 and 2010.[2,3] The exact causes of this epidemic remain unclear. However, building on the concept of the hygiene

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For **national asthma data provided by Statistics Canada** see https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=1310009601

For **national asthma data provided by the US Centers for Disease Control and Prevention** see https://www.cdc.gov/asthma/most_recent_data.htm9

Research in context

Evidence before this study

Asthma is the most prevalent chronic disease of childhood in developed countries; however, the causes of this epidemic remain unclear. Mounting evidence from observational and experimental studies links the development of asthma to dysbiosis of the gut microbiota in early life. Antibiotics, which cause dysbiosis, are a known risk factor for childhood asthma. The frequent use of antibiotics in paediatric patients might also lead to selection for drug-resistant bacteria. Antimicrobial stewardship programmes have been launched in many jurisdictions to optimise the use of antibiotics. However, a formal investigation of the possible association between antibiotic stewardship efforts and contemporary patterns in the incidence of paediatric asthma has not been done. This study was designed to test whether reduced antibiotic prescribing in infants (age <1 year) is associated with a decrease in the incidence of asthma in early childhood (age 1–4 years), and if changes in the gut microbiota in infancy support a plausible biological pathway linking antibiotic exposure to childhood asthma. Following the STROBE guidelines for observational studies, we did a scoping review, searching PubMed for English language studies published from database inception up to Nov 13, 2019, with the terms: (asthma OR wheeze) AND (antibacterial agents OR antimicrobial or anti-infective agents or antibiotic) AND (develop*) AND (early life OR infant OR child*). We identified limitations in 38 directly relevant studies, many of which did not have information on indication for antibiotic use, correction for potential reverse causality or confounding by indication, environmental exposures, genetic factors, or the microbiome.

Added value of this study

To define the relationship between antibiotic use, the gut microbiota, and asthma incidence in children, this study leverages complementary study designs and three independent sources of evidence: (1) population-based administrative data from British Columbia, Canada (population 4·7 million); (2) comprehensive individual-level data from the Canadian Healthy Infant Longitudinal Development (CHILD) prospective birth cohort (n=2644); and (3) a mechanistic investigation of a subsample of the CHILD cohort to assess gut microbiota based on 16S rRNA gene sequencing (n=917). At both the population-level and individual-level, this study shows a robust association between antibiotic exposure in the first year of life and an increased risk of asthma in early childhood. The gut microbiota was found to be a significant mediator between antibiotics and asthma, and the six bacterial taxa that differed in their relative abundance between antibiotic-exposed, asthmatic children and non-exposed, non-asthmatic children have established links with immunomodulatory functions.

Implications of all the available evidence

Our population-level and prospective birth cohort analyses provide evidence that antibiotic exposure in the first year of life is associated with an increased risk of asthma in childhood. A possible mechanism linking antibiotics and asthma is disruption of the structure of the gut microbiota, including depletion of immunoregulatory bacterial taxa. The practical implications of this evidence are: (1) avoiding dysbiosis induced by unnecessary antibiotic therapy might be an effective approach to reducing asthma-related morbidity; and (2) a substantial reduction in observed paediatric asthma incidence in recent years is likely to be an unexpected outcome of prudent antibiotic stewardship.

hypothesis, evidence has strengthened for the protective role of a healthy and diverse community of micro-organisms within and on the body during infancy, with some of the best evidence arising from studies of the gastrointestinal tract and its dense population of gut microbiota.[4,5] An increased relative risk of asthma is seen in infants who are born by caesarean section, who are not breastfed, and who receive antibiotics in infancy.[6–8] These risk factors are thought to cause dysbiosis of the microbial community in the infant by perturbing the population, community succession, and diversity of the infant microbiota. This conceptual framework has been supported by studies in birth cohorts and animal models showing that antibiotic exposure in infancy disrupts the gut microbiota and exacerbates airway inflammation later in childhood.[9,10] One study assessed a subset of patients from the Canadian Healthy Infant Longitudinal Development (CHILD) cohort, and found the absence of key organisms to be associated with a shift towards allergic responses and an increased risk of asthma.[11] In the same study, the protective value of the missing gut microbiota bacteria was verified in a mouse model of asthma.

Although asthma prevalence is high, incidence has decreased in Canada, the UK, and parts of the USA since 2000–05.[2,3,12] Hospitalisations associated with asthma have decreased by 50% in Canada, from 154 per 100 000 of the population aged 0–19 years in 2006, to 75 per 100 000 in 2016.[13] This decrease could be related to improvements in air quality or improved primary care, but whether such changes have been of sufficient magnitude to account for the observed decrease is unclear. Another change at the population level might be exposure to antibiotics, particularly in response to antibiotic stewardship initiatives focused on paediatric prescribing. Receiving one or more courses of antibiotics before the age of 1 year is associated with a 50% increase in the risk of being diagnosed with asthma.[14–16] Rates of antibiotic use in children have decreased in many developed countries in the past 20 years.[17] This decrease

is possibly associated with guidance to limit the use of antibiotics for otitis media, the introduction of conjugated pneumococcal vaccines since 2000, and other antibiotic stewardship efforts. Between 1996 and 2016, a 77% decrease in antibiotic prescriptions in infants (<1 year), from 1342 prescriptions per 1000 babies to 314 per 1000, was documented in British Columbia, Canada, representing a notable reduction in what was once widespread exposure in infancy.

Given the burden of asthma and rapid changes in paediatric antibiotic prescribing, in this study we tested two related hypotheses: whether reduced exposure of infants (aged <1 year) to antibiotics was associated with a decrease in asthma incidence at the population level and individual level within a prospective birth cohort study; and whether changes in the gut microbiota within that cohort would support a plausible biological pathway for such an effect.

Methods

Study design

We did a population-based study of antibiotic prescribing and asthma incidence in British Columbia, Canada, and an analysis of the prospective CHILD birth cohort to assess this association at the individual level, with a mechanistic investigation of the gut microbiota in a subsample of the CHILD cohort. These studies were approved by the institutional review board of the University of British Columbia, Vancouver (approval numbers H09–00650 and H07–03120).

Population antibiotic prescribing and asthma incidence

For the period between 2000 and 2014, prescribing data were obtained from BC PharmaNet, a population-based database hosted by the Government of British Columbia that captures all outpatient dispensing data for the province (database population 4·7 million). We aggregated the number of prescriptions by age, year, sex, local heath authority, and type of antibiotic. We also obtained population estimates for the 91 local health areas of British Columbia and by age group. We calculated the mean percentage of children exposed to one or more courses of antibiotics, mean prescription rates, total number of prescriptions by year, and cumulative percent change in prescription rates. In addition, we obtained aggregate data on annual asthma incidence and prevalence from the British Columbia Ministry of Health Chronic Disease Dashboard using a standard case definition for asthma that integrates a combination of diagnostic codes and asthma-specific drug prescription data from BC PharmaNet.[18] The correlation between antibiotic use and asthma incidence was assessed with the Spearman's rank correlation coefficient. To assess whether a decreasing proportion of infants (<1 year) exposed to at least one course of antibiotics might explain decreasing asthma incidence, we calculated population attributable risk for asthma among children aged 1–4 years and the expected reduction in incidence for relative risk values observed in other studies of the link between antibiotics and asthma (ranging from 1·1 to 3·0.[14–16] Asthma incidence and the mean antibiotic prescribing rate experienced in the first year of life in this cohort were also calculated by year for each of the local health areas in British Columbia. The primary outcome of our population-based study, the association between the rate of antibiotic prescriptions in the first year of life and asthma incidence at age 1–4 years, was estimated with multivariable Poisson regression. Because the mean antibiotic exposure of infants in local health areas ranged from 5% to 66%, we calculated the adjusted incidence rate ratio associated with each 10% increment in infants exposed. Covariates were year, sex, material and social deprivation indices,[19] and mean concentration of fine particulate matter (<2·5 μm [$PM_{2.5}$]) over the year in each local health area. Local health area was included as a random effect to account for unmeasured geographical variability. Statistical analysis of the population-based data was done with STATA software (version 15.1). Further details on how the data were defined and analysed are provided in the appendix (pp 4–5).

For **summary data on antibiotic use of the British Columbia Centre for Disease Control** see http://www.bccdc.ca/health-professionals/data-reports/antimicrobial-resistance-utilization/antimicrobial-utilization-summary

See **Online** for appendix

Prospective CHILD birth cohort

The CHILD study comprises a longitudinal birth cohort (3405 infants in the general cohort) recruited prenatally from four Canadian cities (Vancouver, Edmonton, Winnipeg, and Toronto) between 2008 and 2012.[20] Our analyses included 2644 children clinically assessed for asthma at age 5 years (appendix pp 11, 13). Among those children, a subsample of 917 had 16S rRNA gene sequencing data processed from faecal samples collected at 3 months, 12 months, or 3 and 12 months of age. Antibiotic exposure (type and number of prescriptions) in the first year of life was quantified from questionnaires completed at 3, 6, and 12 months by study families (appendix pp 5–6). The primary outcome for our cohort study was asthma diagnosed by an expert study physician at the clinical assessment at age 5 years. This diagnosis integrated data from a structured clinical history, physical examination, and parental answers to questions regarding respiratory symptoms drawn from the International Study of Asthma and Allergies in Childhood protocol. The appendix (pp 5–6) provides further details on the CHILD cohort and related data. We compared clinical characteristics among children with and without asthma at age 5 years using the Wilcoxon rank sum test for continuous variables and Fisher's exact test for categorical variables in R (version 3.5.1).

For **BC PharmaNet** see https://www.popdata.bc.ca/data/health/PharmaNet

For more on the **local health areas of British Columbia** see https://www2.gov.bc.ca/gov/content/data/geographic-data-services/land-use/administrative-boundaries/health-boundaries

For the **British Columbia Ministry of Health Chronic Disease Dashboard see** http://www.bccdc.ca/health-professionals/data-reports/chronic-disease-dashboard

Characterisation of gut microbiota

The gut microbiota of infants in the CHILD cohort were defined by sequencing the V4 hypervariable region of the

16S rRNA gene in DNA extracted from stool samples collected at 3 and 12 months of age. The methods for DNA processing and sequencing and taxonomic classification have been described previously[11] and are detailed in the appendix (pp 6–7).

Individual antibiotic exposure and asthma incidence

We used multivariable conditional logistic regression (stratified by study recruitment centre) to evaluate the association between systemic antibiotic use in the first year of life and diagnosis of definite asthma, as defined in the CHILD study, at age 5 years (appendix p 5). As a sensitivity analysis, we examined this association but for asthma diagnosed at age 3 years. In another sensitivity analysis, we defined atopic asthma as a phenotype, in children with a definite asthma diagnosis at age 5 years who also had a positive allergy skin prick response by 5 years. The dose–response effect of antibiotics was estimated from the number of courses of antibiotics received (categorised as none, one, two, or three or more courses) in the first year of life. All models were adjusted for study centre, sex, presence of older siblings, mode of delivery, birthweight, season of birth, breastfeeding, ethnicity, tobacco smoke exposure, parental atopy, and exposure to environmental nitrogen dioxide (NO_2), derived from birth charts and questionnaires (appendix p 6). Adjusted odds ratios (aORs) and their 95% CIs were calculated. Missing data were considered missing completely at random after assessing the effect of missing values (appendix p 18) and individuals were removed from the multivariable analysis if they had a missing value in any covariates. Potential biases associated with confounding by indication and reverse causation were investigated, in which we excluded: children who received antibiotics for respiratory symptoms; children with a diagnosis of wheeze in the first year of life; or both of these groups (appendix p 6). All statistical analyses of outcomes in the CHILD cohort were done with R software (version 3.5.1).

Gut microbiota, asthma, and antibiotics

We used conditional logistic regression and linear mixed effects models with the study centre as a random intercept to examine the associations between gut microbiota α-diversity and asthma, and between antibiotic use and gut microbiota diversity, using the α-diversity indices of Faith's phylogenetic diversity (PD) and Chao1 as measures of the diversity of bacterial taxa within our sample. The dose–response and time-to-effect of antibiotics were also estimated according to the time of first antibiotic exposure (categorised as any use between 2 days to 3 months, 3–6 months, 6–9 months, and 9–12 months). All analyses were done with adjustment for the same covariates (appendix p 6). To identify bacterial taxa that might be driving significant differences in asthma incidence and antibiotic exposure, the R packages DESeq2 and edgeR were applied (appendix p 7).[21,22]

Structural equation modelling and mediation analysis

We used structural equation modelling to examine both direct and indirect causal relationships,[23] testing the hypothesis that any effect of antibiotics would occur via disruption of the gut microbiota, driving the subsequent development of asthma. Using a sequential approach for the latent variable, we first examined mediation via an unsupervised measure of microbiota diversity (in terms of α-diversity and β-diversity), and then repeated this analysis using a latent variable that included both microbiota diversity and any amplicon sequence variants (ASVs) that were modified by antibiotic exposure. The appendix (p 7) provides full details on the modelling strategy and analysis.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. HS, DLYD, SET, FSLB, GLW, MBA, ABB, PJM, TJM, MRS, CP, RCTB, PS, and BBF had access to raw data from the CHILD cohort; and DMP, AAM, and DR had access to the provincial ecological raw data. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

At the population level in British Columbia, from 2000 to 2014, the annual incidence of asthma in children (aged 1–4 years) showed an absolute decrease of 7·1 new diagnoses per 1000 children, from 27·3 (26·8–28·3) per 1000 children, to 20·2 (19·5–20·8) per 1000 children (a relative decrease of 26·0%; figure 1A). This rate of decrease in asthma incidence represents 1264 fewer cases each year in this age group. For children aged 1–4 years in 2000, the corresponding mean annual rate of antibiotic prescribing during their first year of life was 1253·8 prescriptions (1219·3–1288·9) per 1000 infants (aged <1 year). This rate decreased by 764·7 prescriptions per 1000 infants, to 489·1 (467·6–511·2) per 1000 infants, by 2014 (a relative decrease of 61·0%; figure 1A). The mean proportion of children exposed to one or more courses of antibiotics before the age of 1 year similarly decreased by 34·8%, from 66·9% (5·2) to 32·1% (2·6) during the same period, representing a cumulative relative decrease in annual antibiotic prescription rate of 60·9% (figure 1B, C). In addition to the change in disease epidemiology represented by the decrease in incidence, we also found a decrease in asthma prevalence during the study period (appendix p 12). Asthma incidence in children and antibiotic use in the first year of life were strongly correlated (Spearman's r=0·81; p<0·0001). Amoxicillin was the most frequently prescribed antibiotic for infants across the study period, making up 10 537 (65·7%) of 16 034 antibiotic prescriptions in 2014 (appendix p 14).

Multiple factors, including changes in the environment,

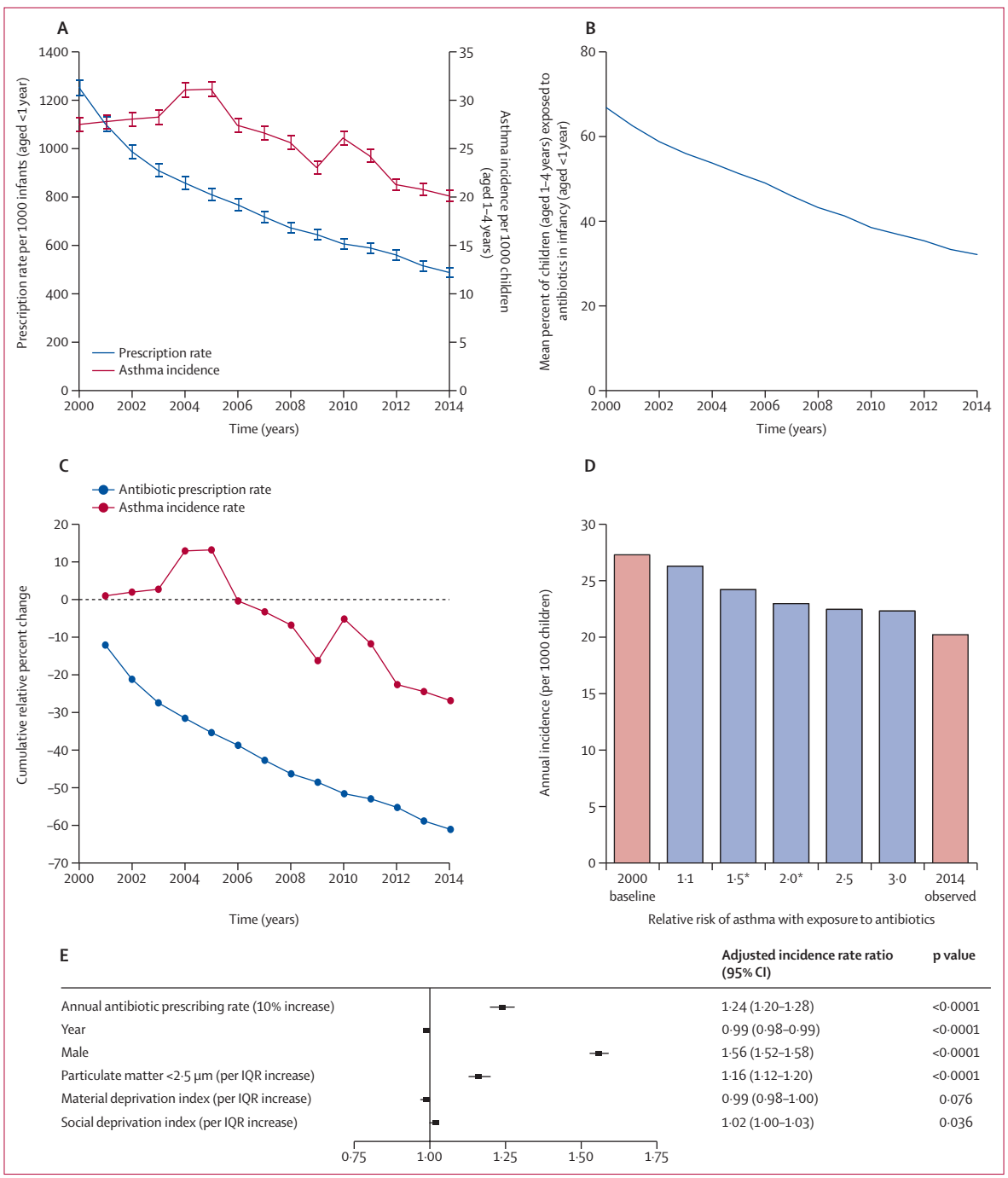


Figure 1: **Population-level study of children (aged 1–4 years) in British Columbia, Canada**
(A) Asthma incidence in children from 2000 to 2014, and average mean antibiotic prescription rate within this population in the first year of life. (B) Mean percentage of children who received one or more antibiotic prescriptions in their first year of life. (C) Cumulative relative percent change in antibiotic prescriptions and asthma incidence rates. (D) Modelling of expected asthma incidence in 2014, given the observed reduction in the proportion of children exposed to antibiotics under various assumptions for the relative risk of asthma related to antibiotic exposure. (E) Multivariable Poisson regression of factors associated with asthma incidence at the level of local health areas (n=91) in British Columbia. *The relative risk range (1·5–2·0) most commonly reported in the literature.[14–16]

might have contributed to the marked decrease in the incidence of early childhood asthma that we observed. Using the population attributable risk method (appendix p 4), we made predictions about the expected asthma incidence after the observed reduction in exposure to antibiotics (figure 1D). We modelled the expected incidence with a range of relative risk scores on the association between antibiotic exposure and asthma. In

one scenario, taking the actual relative risk of asthma to be 2·0 following exposure to antibiotics, we would predict the annual asthma incidence to decrease from 27·3 new diagnoses per 1000 children to 22·9 per 1000 children; the observed incidence actually decreased further to 20 per 1000 children by 2014.

To understand the association between antibiotic exposure in infancy and asthma in early childhood at a finer spatial scale and to account for geographical variability, we examined data segregated according to the 91 local health areas within British Columbia. The adjusted incidence rate ratios (aIRRs) predicting asthma incidence in children aged 1–4 years are depicted in figure 1E. With this approach we confirmed a significant association between annual antibiotic prescribing rate and asthma incidence (aIRR 1·24 per 10% absolute increase in antibiotic prescribing [95% CI 1·20–1·28]; p<0·0001). Among other variables included in the model, male gender, and $PM_{2·5}$ showed significant associations.

The CHILD cohort study allowed us to further define the relationship between antibiotic exposure in infancy and childhood asthma at the individual level. Among the sample of 2644 children clinically assessed for asthma at age 5 years across the four study sites (Vancouver [n=614], Edmonton [n=551], Winnipeg [n=901], and Toronto [n=578]), 164 (6·2%) were diagnosed with definite asthma. 462 (17·5%) children had received systemic antibiotics in the first year of life following hospital discharge (appendix p 13). Similar to our findings for provincial-level prescribing, aminopenicillins (amoxicillin, ampicillin, and amoxicillin–clavulanate) represented 393 (59%) of the total 670 prescriptions (appendix p 15). Outpatient antibiotic use within the first year of life, ethnicity, mode of delivery, sex, and parental atopy were significantly associated with asthma diagnosis at age 5 years (appendix p 16). After adjusting for covariates and excluding observations without data (considered missing at random; appendix p 18), the aOR for the association between outpatient antibiotic exposure in the first year of life and asthma diagnosis at age 5 years was 2·54 (95% CI 1·70–3·78; p<0·0001; data not shown). This relationship was maintained in the subset of children with atopic asthma (ie, allergic sensitisation plus asthma at age 5 years; aOR 2·03 [1·14–3·63]; p=0·016) and more generally in children presenting with sensitisation at age 5 years (1·41 [1·04–1·92]; p=0·029; appendix p 22). Additionally, we confirmed this association for asthma diagnosed at age 3 years in our sensitivity analysis (aOR 1·69 [1·04–2·75]; p=0·035) whereby the case ascertainments were temporally aligned across the population in British Columbia (age 1–4 years; data not shown) and the CHILD cohort samples (age 3 years; appendix p 17).

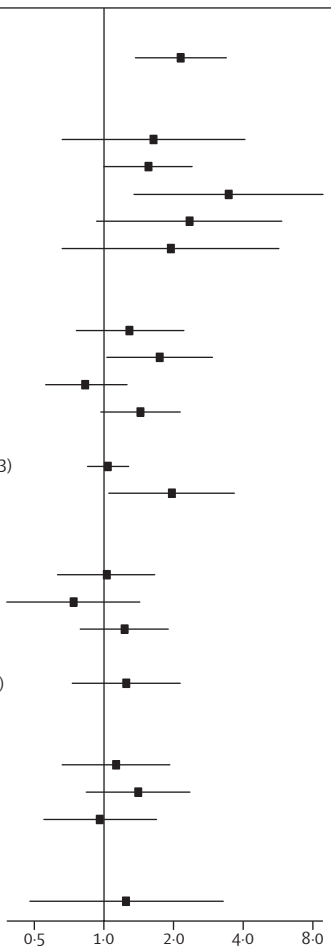
Because respiratory infections could be both an indication for antibiotic use and a risk factor for asthma, to address this risk of confounding by indication, we excluded 95 children who received antibiotics for respiratory symptoms, which gave an aOR of 2·15 (1·37–3·39; p=0·0009; figure 2A). A dose–response effect was indicated by an increase in the risk of asthma with the number of courses of antibiotics; 114 (5·2%) of 2182 children unexposed to antibiotics had asthma by age 5 years, compared with 23 (8·1%) of 284 exposed to one course, five (10·2%) of 49 exposed to two courses, and six (17·6%) of 34 exposed to three or more courses (aOR 1·44 [1·16–1·79]; p=0·0008; figure 2B). The aOR for diagnosis of asthma in children exposed to one course of antibiotics was 1·93 (1·15–3·26; p=0·013), which increased to 3·25 (1·05–10·08; p=0·041) in children exposed to three or more courses (appendix p 19). To address the possibility of reverse causation, we excluded 272 children who had a diagnosis of wheeze up to age 1 year, and found that antibiotic use in the first year of life was associated with a doubling of the risk of a diagnosis of asthma at age 5 years (aOR 1·99 [1·20–3·30]; p=0·0075; appendix p 20). When both of the potential biases of reverse causation and indication for respiratory conditions were considered, we observed a slightly attenuated risk estimate compared with analysis of each bias in isolation (aOR 1·66 [0·94–2·92]; p=0·080; appendix p 21), albeit in a much smaller sample due to the exclusion of children with wheeze and children prescribed antibiotics for respiratory conditions in their first year of life.

To dissect the mechanisms by which antibiotics in infancy might influence the risk of developing asthma, we examined the gut microbiota in a subsample of our cohort from the CHILD study. In 917 children with available 16S rRNA sequencing data at 3 months or 1 year, the association between antibiotic use in the first year of life and the risk of an asthma diagnosis at age 5 years was maintained (aOR 1·87 [1·05–3·33]; p=0·034). As expected, α-diversity index values (PD and Chao1) increased significantly from age 3 months to 1 year (figure 3A). In children diagnosed with asthma at age 5 years, α-diversity at age 1 year was lower than in those children without asthma (figure 3B). The association between reduced microbiota diversity at age 1 year and diagnosis of asthma at age 5 years was further confirmed after controlling for potential confounders, whereby we observed a 32% reduction in asthma risk associated with an IQR increase (25th to 75th percentile) in the Chao1 index (aOR 0·68 [0·46–0·99]; p=0·046; figure 3C).

In the CHILD cohort, we subsequently examined the effect of antibiotic exposure on the gut microbiota. Although the α-diversity of stool samples at age 1 year was not different when outpatient antibiotic exposure was considered as a binary outcome (exposure or no exposure; figure 4A), important differences emerged when we considered both the number of courses and the timing of antibiotic prescriptions. Increasing courses of antibiotics in the first year of life were associated with significantly

A

	Asthma	No asthma	Adjusted odds ratio	p value
Participants	118	1829	..	..
Antibiotic use by age 1 year	29 (24·6%)	260 (14·2%)	2·15 (1·37 to 3·39)	0·0009
Ethnicity				
White Caucasian	60 (50·8%)	1202 (65·7%)	Ref	..
East Asian	6 (5·1%)	65 (3·6%)	1·64 (0·66 to 4·08)	0·29
Multiracial	36 (30·5%)	431 (23·6%)	1·56 (1·00 to 2·41)	0·048
South Asian	6 (5·1%)	34 (1·9%)	3·47 (1·35 to 8·88)	0·0095
Southeast Asian	6 (5·1%)	47 (2·6%)	2·35 (0·93 to 5·89)	0·069
Other	4 (3·4%)	50 (2·7%)	1·95 (0·66 to 5·72)	0·23
Mode of delivery				
Vaginal	77 (65·3%)	1405 (76·8%)	Ref	..
Caesarean section with labour	20 (16·9%)	223 (12·2%)	1·29 (0·76 to 2·22)	0·35
Caesarean section without labour	21 (17·8%)	201 (11·0%)	1·88 (1·11 to 3·18)	0·018
≥1 older sibling	51 (43·2%)	866 (47·3%)	0·83 (0·56 to 1·26)	0·39
Male	74 (62·7%)	963 (52·7%)	1·44 (0·97 to 2·14)	0·069
Birthweight Z score				
Median (range)	−0·1 (−2·0 to 2·5)	−0·1 (−3·1 to 4·3)	1·04 (0·85 to 1·28)	0·67
Parental atopy	106 (89·8%)	1475 (80·6%)	1·97 (1·05 to 3·67)	0·034
Breastfeeding status at 6 months				
None	26 (22%)	383 (20·9%)	Ref	..
Partial	75 (63·6%)	1104 (60·4%)	1·03 (0·63 to 1·66)	0·92
Exclusive	17 (14·4%)	342 (18·7%)	0·74 (0·38 to 1·43)	0·37
Tobacco smoke exposure up to age 1 year	34 (28·8%)	466 (25·5%)	1·23 (0·79 to 1·9)	0·36
NO_2 (parts per billion) in year 1, per IQR increase				
Median (range)	1·2 (0·3 to 3·4)	1·1 (0·1 to 3·4)	1·25 (0·73 to 2·14)	0·42
Season of birth				
Spring	29 (24·6%)	514 (28·1%)	Ref	..
Summer	29 (24·6%)	459 (25·1%)	1·13 (0·66 to 1·93)	0·66
Autumn	35 (29·7%)	410 (22·4%)	1·41 (0·84 to 2·36)	0·20
Winter	25 (21·2%)	446 (24·4%)	0·96 (0·55 to 1·69)	0·89
Urban *vs* rural living				
Urban	113 (95·8%)	1721 (94·1%)	Ref	..
Rural	5 (4·2%)	108 (5·9%)	0·99 (0·38 to 2·61)	0·99



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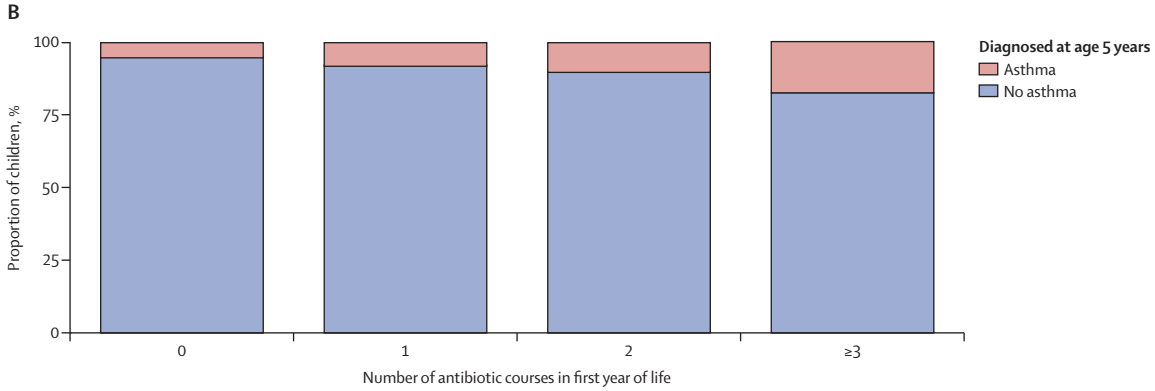
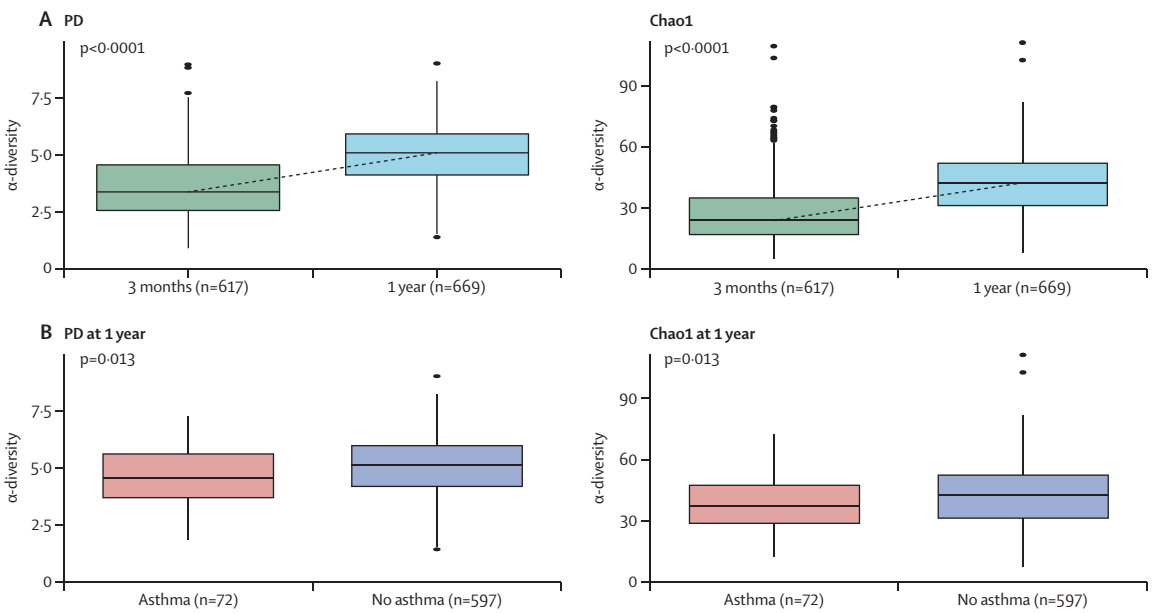


Figure 2: **Individual-level study of children from the Canadian Healthy Infant Longitudinal Development prospective birth cohort**
(A) Multivariable conditional logistic regression showing antibiotic use by age 1 year (for non-respiratory indications) and other factors associated with asthma diagnosis at age 5 years. Group totals account for missing data across the risk factors (data missing: ethnicity, n=32 [one in asthma group and 31 in no asthma group]; mode of delivery, n=28 [one and 27]; presence of older sibling, n=42 [one and 41]; birthweight Z-score, n=150 [nine and 141]; parental atopy, n=69 [four and 65]; breastfeeding at 6 months, n=63 [two and 61]; tobacco smoke exposure, n=284 [12 and 272], NO_2 in year 1, n=14 [one and 13]; season of birth, n=2 [both non-asthmatic]; urban versus rural, n=111 [seven and 104]**)**. Data were considered missing completely at random after assessing the effect of missing values (appendix p 18). (B) Asthma prevalence at age 5 years stratified by the number of outpatient antibiotic courses received in the first year of life. NO_2=nitrogen dioxide.



C

	Asthma	No asthma	Adjusted odds ratio	p value
Participants*	63	507	..	..
Chao1 at 1 year (IQR)				
Median (range)	1·8 (0·6 to 3·5)	2·1 (0·4 to 5·3)	0·68 (0·46 to 0·99)	0·046
Ethnicity of child				
White Caucasian	31 (49·2%)	345 (68·0%)	Ref	..
East Asian	3 (4·8%)	18 (3·6%)	1·68 (0·44 to 6·41)	0·45
Multiracial	23 (36·5%)	118 (23·3%)	1·95 (1·06 to 3·58)	0·032
South Asian	1 (1·6%)	5 (1·0%)	1·92 (0·19 to 19·18)	0·58
Southeast Asian	4 (6·3%)	14 (2·8%)	2·65 (0·76 to 9·22)	0·13
Other	1 (1·6%)	7 (1·4%)	1·86 (0·20 to 17·19)	0·58
Mode of delivery				
Vaginal	42 (66·7%)	390 (76·9%)	Ref	..
Caesarean section with labour	10 (15·9%)	53 (10·5%)	1·64 (0·73 to 3·65)	0·23
Caesarean section without labour	11 (17·5%)	64 (12·6%)	1·55 (0·72 to 3·33)	0·27
≥1 older sibling	29 (46·0%)	242 (47·7%)	1·09 (0·60 to 1·98)	0·78
Male	40 (63·5%)	285 (56·2%)	1·27 (0·72 to 2·24)	0·41
Birthweight Z score				
Median (range)	−0·2 (−2·0 to 1·7)	0 (−2·5 to 3·7)	0·85 (0·63 to 1·14)	0·28
Parental atopy	54 (85·7%)	404 (79·7%)	1·36 (0·62 to 2·97)	0·45
Breastfeeding status at 6 months				
None	16 (25·4%)	100 (19·7%)	Ref	..
Partial	37 (58·7%)	314 (61·9%)	0·74 (0·38 to 1·46)	0·39
Exclusive	10 (15·9%)	93 (18·3%)	0·67 (0·27 to 1·64)	0·38
Tobacco smoke exposure up to age 1 year	16 (25·4%)	122 (24·1%)	1·05 (0·55 to 1·98)	0·89
NO_2 (parts per billion) in year 1, per IQR increase				
Median (range)	1·2 (0·3 to 3·1)	1·1 (0·1 to 3·1)	1·55 (0·66 to 3·64)	0·32
Season of birth				
Spring	18 (28·6%)	135 (26·6%)	Ref	..
Summer	20 (31·7%)	128 (25·2%)	1·21 (0·60 to 2·45)	0·59
Autumn	12 (19·0%)	121 (23·9%)	0·68 (0·3o to 1·52)	0·34
Winter	13 (20·6%)	123 (24·3%)	0·72 (0·33 to 1·58)	0·42

decreased α-diversity (figure 4B). Furthermore, the stool of infants who received outpatient antibiotics before 3 months of age had significantly reduced α-diversity at age 1 year (figure 4C). Applying a mixed linear effects model, antibiotic use was significantly associated with reduced α-diversity at age 1 year (Chao1 fixed effects coefficient [β] –0·24; p=0·0028; appendix p 23). This effect was more pronounced if systemic antibiotics were taken before 3 months of age (–0·69; p=0·0007), than between age 9 and 12 months (–0·27; p=0·019; appendix p 23).

We applied an integrative bioinformatics strategy to determine whether specific bacterial ASVs within stool samples at age 1 year were linked with changes in microbiota diversity in children with asthma and in those exposed to antibiotics in infancy. We assessed outputs of DESeq2 and edgeR, whereby sequencing batch, study centre, sex, presence of older siblings, mode of delivery, birthweight, birth season, breastfeeding, ethnicity, tobacco smoke exposure, parental atopy, and environmental NO_2 were controlled. With these approaches we identified six ASVs associated with asthma and antibiotic exposure, common to both bioinformatics approaches (appendix pp 24–25). The six selected ASVs mapped to the Rikenellaceae family (one variant), and five genera and four species (five variants), including *Faecalibacterium prausnitzii*, *Roseburia*, *Ruminococcus bromii*, and *Clostridium perfringens* (figure 5A). Consistent with our observations that an overall loss of microbial diversity was associated with both asthma and antibiotic exposure, of the ASVs identified, all but one were significantly reduced in association with these parameters.

To test our hypothesis that any effect of antibiotics would occur via disruption of the gut microbiota, we used a structural equation modelling approach to assess alternative explanations for a role of the gut microbiota in associations between antibiotic exposure in infancy and early childhood asthma. We found that the gut microbiota at age 1 year, as reflected by α-diversity and β-diversity measures, was a significant mediator between outpatient antibiotic exposure in the first year of life and asthma diagnosis at age 5 years (after adjusting for covariates, effect estimate β=0·07, p=0·028; data not shown), whereas no significant direct effect was evident between antibiotics and asthma (appendix pp 28–29). Adding ASVs modified by antibiotic exposure to the latent variable confirmed the mediating effect (after adjusting for covariates, β=0·08, p=0·027; figure 5B and appendix pp 28–29).

Figure 3: **Association between the gut microbiota and asthma**
(A) Changes in α-diversity of the gut microbiota from age 3 months to 1 year. Wilcoxon p values are shown. (B) Differences in α-diversity between asthmatic and non-asthmatic children. (C) Multivariable logistic regression showing Chao1 index (IQR change) and other risk factors associated with asthma at age 5 years. PD=Faith's phylogenetic diversity. NO_2=nitrogen dioxide. *All those with microbiota samples processed at 1 year.

Discussion

In our population-based study of 4·7 million people in British Columbia, we identified a 26·0% decrease in asthma incidence between 2000 and 2014 in young children, which correlated with a large decrease in the corresponding antibiotic prescription rate in infancy. The delay between reduced prescription rates and decreased asthma incidence is likely to reflect the 4 years required to collect data on a full cohort of children aged 1–4 years. Putting the reduction in incidence into a broader context, if a decrease of this magnitude occurred in Europe, a predicted 147 000 fewer asthma cases in early childhood would have occurred per year in Europe over the same period.

Given the value of replication with different study designs, we explored the relationship between antibiotic exposure in infancy and early childhood asthma at the individual level in a prospective birth cohort of children. We observed that antibiotic use in the first year of life was associated with almost double the risk of an asthma diagnosis at age 5 years. A concern with some earlier studies linking antibiotic exposure in early life to childhood asthma has been that bias from confounding by indication and reverse causation might explain the positive associations observed.[24] A strength of the prospective study design of the CHILD cohort is that we could address both of these risks. We found that the association between outpatient antibiotic exposure before age 1 year and asthma diagnosis at age 5 years was maintained after excluding either children who received antibiotics for respiratory indications or children who had a diagnosis of wheeze in the first year of life. Although we explicitly acknowledge that we are reporting associations, and further mechanistic studies and carefully designed trials are necessary, when taken together, our studies largely satisfy the Bradford-Hill criteria for investigating causality in epidemiological studies (appendix p 30).[25] Specifically, in considering the link between antibiotic exposure in infancy and later development of asthma, our data show consistency (through replication in both our population and cohort studies), biological gradient (given the positive relationship with increasing antibiotic courses and asthma), and plausibility (with evidence that the association is significantly mediated by variation in the structure of the infant gut microbiota).

In addition to reduced rates of antibiotic prescribing, other factors such as reductions in air pollution and varying immigration rates by country of origin (ie, the healthy immigrant effect)[26] are likely to have contributed to the observed associations. In our study, we modelled expected incidence with a range of previous relative risk estimates. This method implied that reduced use of antibiotics in young children was a major factor, but not the sole one, contributing to decreased asthma incidence,

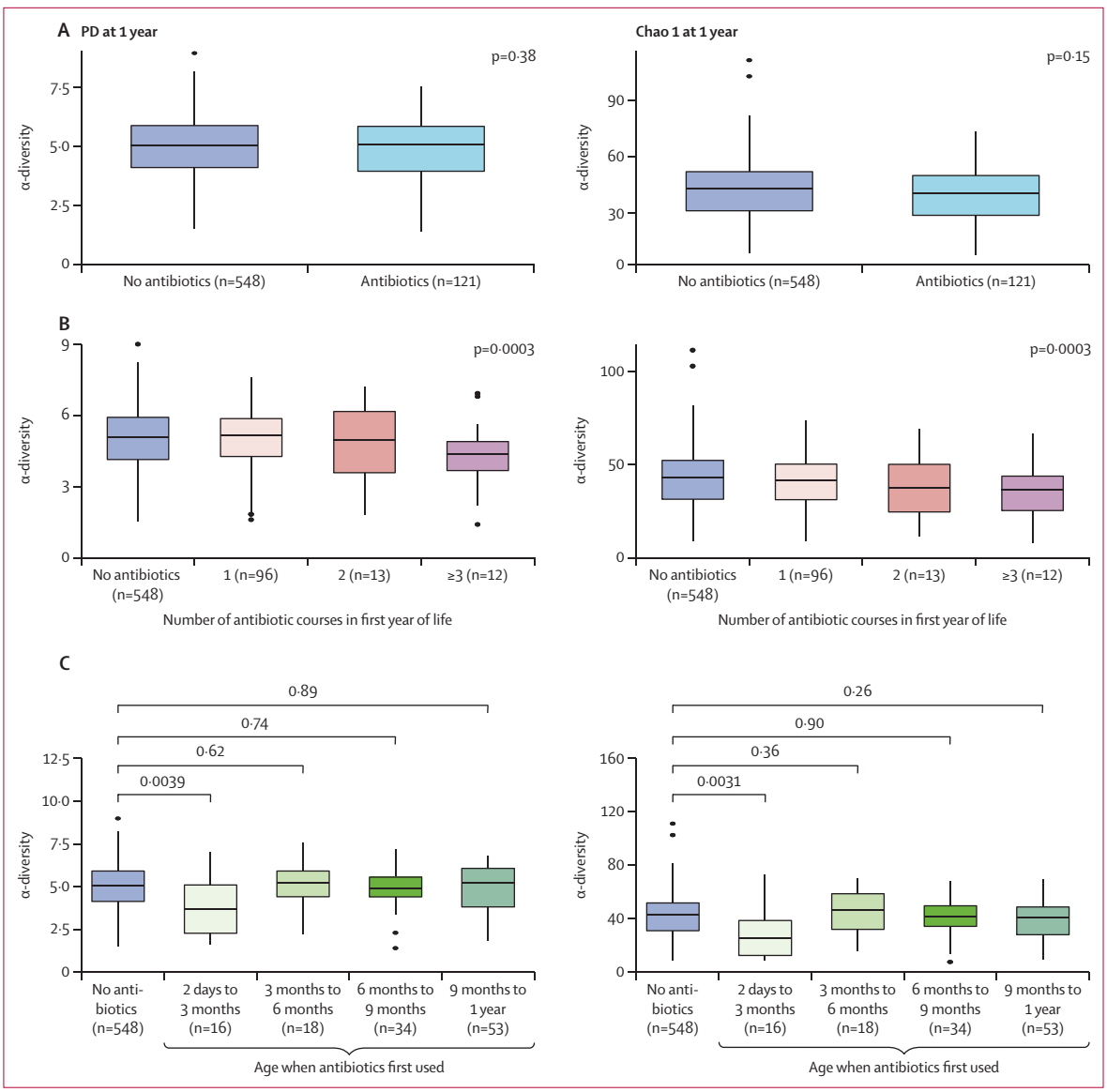


Figure 4: **Association between the gut microbiota and antibiotics**
(A) Differences in gut microbiota α-diversity at 1 year in children exposed or not exposed to antibiotics. (B) Differences in gut microbiota α-diversity at 1 year according to the number of courses of antibiotics received in infancy. (C) Differences in gut microbiota α-diversity at 1 year according to the age at which antibiotics were first used. Wilcoxon p values are shown. PD=Faith's phylogenetic diversity.

and that air pollution, as quantified by the concentration of $PM_{2·5}$, was an important independent predictor of asthma at the population level. Therefore, improved air quality might be one factor that explains why the incidence of asthma decreased more than predicted on the basis of reduced antibiotic prescriptions alone. Furthermore, our analyses indicated an effect of south Asian ethnicity, possibly indicating a genetic susceptibility and environmental interaction on the risk of disease, although our study was not designed to conclude on the influence of ethnicity.

A strength of our study is that we offer plausible mechanisms linking antibiotic exposure in early life to the subsequent development of asthma. In addition to a reduction in bacterial diversity, the gut microbiota of children in the CHILD cohort who received antibiotics and developed asthma exhibited reductions in five key bacterial taxa. Of the bacterial species we found to be depleted in association with antibiotic exposure and asthma, two have intriguing mechanistic links to asthma, via the production of immunomodulatory short-chain fatty acids (SCFAs). *R bromii* is a key species of the gut microbiota, with a superior ability to degrade the group of carbohydrate polymers that form resistant starch, which cannot be digested by human enzymes.[27] Breakdown products from resistant starch are cross-fed to fermentative bacteria that

produce SCFAs, such as *F prausnitzii*, which is an anti-inflammatory commensal bacteria that produces butyrate and is depleted in the stool of asthmatic adults and infants who develop asthma.[28–30] Furthermore, sporulation of *C perfingens* (the only ASV increased in relative abundance in antibiotic-exposed asthmatic children in this study) has been shown to be inhibited by SCFAs,[31] further supporting the observation that loss of a fermentative gut microbiome early in life might be a risk factor for the development of asthma. Together these data emphasise the potential importance of immunomodulatory metabolites derived from bacteria, and how depletion of specific species could skew immune development toward an allergic phenotype. A combination of metagenomic and metabolomics data will be required to confirm our findings.[32]

Our study was designed to address several gaps in the evidence base and limitations of previous studies, but also raises future research questions. We are currently designing a retrospective population-based cohort study that will analyse linked administrative data on all infants born in British Columbia between 2001 and 2011. The aim of this study will be to evaluate the effect of reduced antibiotic use on asthma, allergic rhinitis, and atopic dermatitis, thereby offering an insight into subpopulations treated for different indications. Given the challenges inherent to diagnosing asthma in young children, we included children in the CHILD cohort diagnosed with possible asthma in our no asthma group, as misclassification by this route would produce a conservative bias. Although we were able to leverage the full power of the CHILD cohort study across four Canadian provinces, granular population data to inform ecological analysis were only readily accessed from British Columbia. However, antibiotic use in Alberta, Manitoba, and Ontario has followed a similar pattern.[33] Equally, asthma hospitalisation rates have decreased significantly in these provinces over 2006–16,[13] which supports the view that antimicrobial stewardship, especially in infancy, can prevent gut microbiota dysbiosis and help to re-establish the diverse ecology of microbes required for healthy immune development.[34] Although our study was deliberate in its focus on the effect of antibiotics on the bacterial composition of the gut microbiota, the gut also contains fungal communities that might be modulated by antibiotics and potentially involved in the pathogenesis of asthma.[29] Furthermore, other bacterial communities within the body that were not sampled in this study, such as the airway microbiota,[35] are likely to be modified by antibiotic exposure. Although we present a compelling combination of data from three complementary study designs in support of a putative causal link between antibiotic exposure in infancy and asthma in early childhood, these associations need to be clarified by a combination of animal studies and clinical trials that focus on reversing dysbiosis following antibiotic treatment.

In conclusion, in our population-level and birth cohort analyses, we found evidence that antibiotic exposure in the first year of life is associated with an increased risk of asthma in childhood. Our findings are most consistent with a mechanism by which antibiotics reduce the diversity of the microbiota and skew the developing immune system toward atopic responses. This work opens up the question of whether novel therapies should be used to

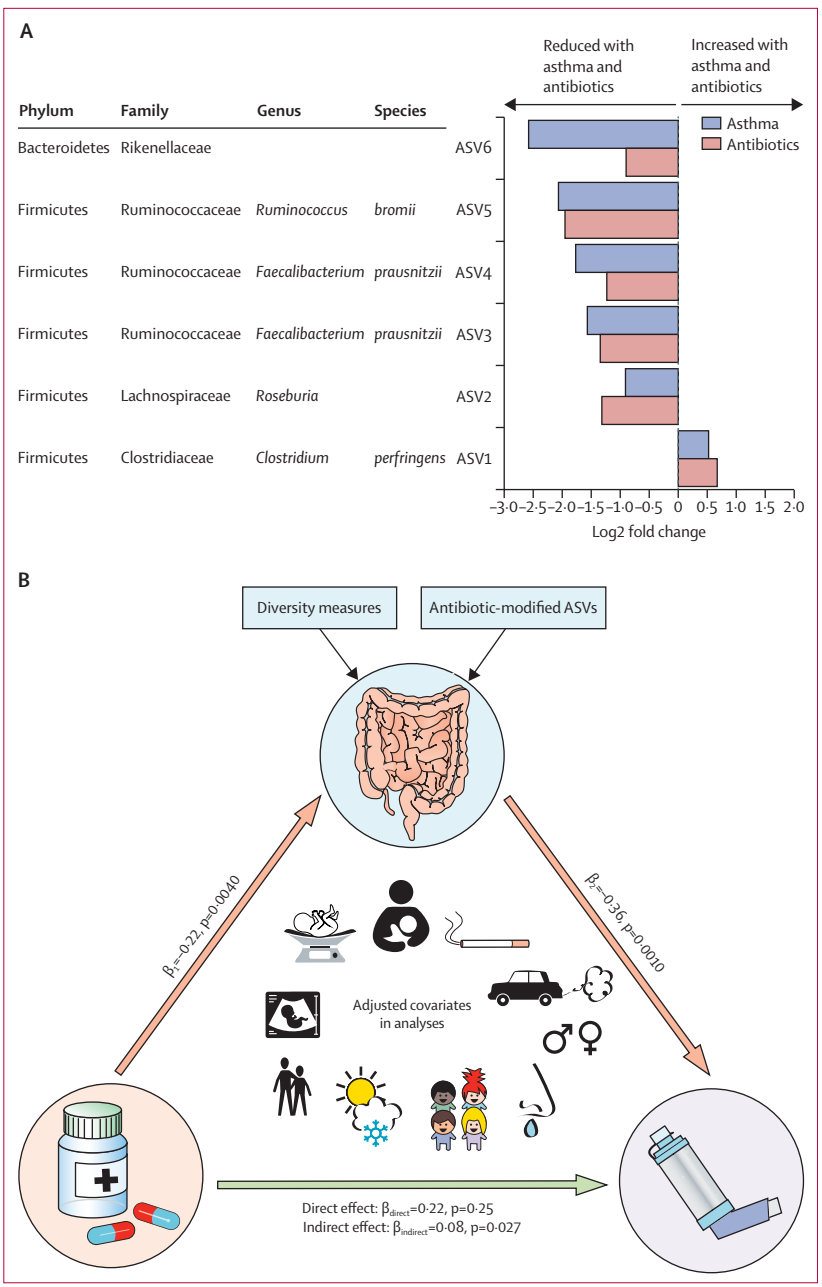


Figure 5: **Gut microbiota structure as a link between antibiotic exposure and asthma**
(A) Bar plot of log2 fold change between groups based on raw counts after normalisation to sequencing depth and composition. (B) Standardised regression coefficients in our structural equation modelling for the relationship between antibiotic use before age 1 year and asthma diagnosis at age 5 years mediated by the gut microbiota (all standardised regressions shown are controlled for sex, presence of older siblings, mode of delivery, birthweight, birth season, breastfeeding, ethnicity, exposure to smoking, parental atopy, and nitrogen dioxide exposure). ASV=amplicon sequence variant.

maintain microbiota diversity following antibiotic exposure. However, we conclude that the shortest path to reducing asthma-related morbidity is by avoiding dysbiosis induced by unnecessary antibiotic therapy. Furthermore, the substantial reduction in paediatric asthma incidence observed in recent years is an unexpected yet plausible benefit of prudent antibiotic stewardship.

Contributors

DMP, HS, BBF, and SET conceived the synthesis of three levels of evidence. DMP, DR, AAM, and FM conceived and did the population-level studies. PS, SET, PJM, ABB, MBA, TJM, and MRS designed and did the CHILD cohort study. DMP, AAM, DR, HS, DLYD, CR, RCTB, CP, LTS, FSLB, GLW, SET, and BBF planned the analyses and interpreted the data. All authors contributed to writing and reviewing the paper, have approved it for submission, and agree to being accountable for its content.

Declaration of interests

HS, DYLD, RCTB, CP, BBF, and SET are listed as inventors by the University of British Columbia (Vancouver, BC, Canada) for a US provisional patent application (62/927,248 bacterial compositions and methods altering gut microbiota), related to this work. All other authors declare no competing interests.

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