frontiers | Research Topics

Preventing childhood asthma: the neglected impact of existing public health interventions

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Published in
Frontiers in Allergy
Frontiers in Public Health
Frontiers in Pediatrics

ISSN 1664-8714
ISBN 978-2-8325-6429-5
DOI 10.3389/978-2-8325-6429-5

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Preventing childhood asthma: the neglected impact of existing public health interventions

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Citation
Patrick, D. M., Turvey, S., Azad, M. B., Zimmermann, P., Dramowski, A., Lishman, H., eds. (2025). *Preventing childhood asthma: the neglected impact of existing public health interventions*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-8325-6429-5

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frontiers | Frontiers in Allergy

TYPE Editorial
PUBLISHED 23 May 2025
DOI 10.3389/falgy.2025.1621332

OPEN ACCESS

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RECEIVED 30 April 2025
ACCEPTED 13 May 2025
PUBLISHED 23 May 2025

CITATION
Patrick DM, Turvey SE, Azad MB, Zimmermann P, Dramowski A and Lishman H (2025) Editorial: Preventing childhood asthma—the neglected impact of existing public health interventions.
Front. Allergy 6:1621332.
doi: 10.3389/falgy.2025.1621332

Editorial: Preventing childhood asthma—the neglected impact of existing public health interventions

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KEYWORDS

asthma, atopic disease, antibiotics, human milk, microbiota, population

Editorial on the Research Topic
Preventing childhood asthma - the neglected impact of existing public health interventions

Asthma is the most prevalent chronic disease of childhood in high income countries, with prevalence rising steeply during the late 20th century, a pattern now echoed in some middle-income countries (1, 2). How can this vast burden of illness be reduced? Current strategies focus appropriately on environmental factors such as indoor and outdoor air quality, and ensuring access to effective medical treatment (3). This series summarizes growing evidence that exposure to microbes and human breastmilk during infancy shape the immune system and influence lifelong risk for atopic disease, including asthma. Such evidence begs the question: can existing public health interventions, such as reducing unnecessary antibiotic exposure during infancy and promoting breastfeeding, contribute to reversing the global pandemic of asthma and atopic disease?

Hildebrand and Adamko open the series by reviewing the changing epidemiology and distribution of asthma and allergy. The global burden of asthma and allergic disease has remained high since the late 20th century, but uneven distribution of prevalence between countries and across socioeconomic contexts highlights the complexity of early life exposures and their interaction with genetic predisposition for atopic disease.

Donald and Finlay neatly summarize crucial insight from experimental models that provide evidence of a link between antibiotic exposure, disrupted development of infant gut microbiota and subsequent risk of atopic disease. Mice without microflora have a higher risk of atopic disease, but proper immune function can be rescued by replacement of missing bacterial taxa in a critical early-life window.

Donald and Finlay's second contribution is a detailed review of known mechanisms of immune mediation by the developing gut microbiota and its links to lifelong atopic disease risk. The human gut is the major point of contact between the immune system and the environment. Specific microbes produce metabolites, including short chain fatty acids, that "train" the immune system away from atopic responses by regulating a variety of processes, including T_{reg} development. Thus, there is a plausible pathway by which early life exposures imprint on life-long immune responses.

The global literature points to the importance of a number of pre- and perinatal exposures in predicting asthma risk, and in such studies, receipt of antibiotics during infancy has a large effect (4). Wurm et al. provide a systematic review of the effect of antibiotics on the intestinal microbiota in children. Antibiotic therapy during the first year of life clearly disrupts the gut microflora during infancy and childhood, including taxa implicated as beneficial in preventing atopic disease. Moreover, the effects on microbiota and atopic disease risk seem worse for macrolides than aminopenicillins; an important observation, because health systems are contemplating broad use of prophylactic macrolides for children in regions with possible benefit for infant mortality (5).

Brockway summarizes the potential for human milk to mitigate antibiotic-mediated atopic disease risk by preventing or repairing antibiotic-associated disruption of the infant gut microbiota. Human milk oligosaccharides, while not metabolized by the infant, favour the growth of *Bifidobacterium infantis* and other beneficial bacteria. Human milk feeding to at least six months is already recommended, but there is variation in the practice globally and limited availability of donor human milk for families unable to breastfeed.

Loutet provides a detailed study of factors associated with breastfeeding in Bangladesh, outlining the individual and system challenges in lower resource settings. Providing human milk to an infant is harder where both parents need to work, where public breastfeeding is less accepted and in the face of intensive formula marketing. In settings where antibiotic use in infancy and caesarean section rates are also high, a compounding effect on microbiome disruption must be considered when investigating the impact on atopic disease outcomes. Stronger system-level supports are required to overcome deficits in education, services and policies to promote and protect breastfeeding.

An important criticism of any theory linking antibiotics to atopic disease is the question of confounding by indication: what if the infections that caused antibiotic prescription are themselves the cause of asthma? Medeleanu et al. analyze data from a prospective birth cohort to address the interface with early-life respiratory infections and conclude that while those infections may confer independent risk for asthma, they do not explain the impact of antibiotic exposure. Further, antibiotics are associated with an array of atopic outcomes, not just asthma, speaking to the importance of a common immunomodulatory pathway.

This issue also includes articles that address additional observations about the complex pathogenesis of atopic asthma. Keleb et al. speak to the importance of outdoor environmental exposures by contributing a global systematic review and meta-analysis of the effect of pesticide exposure on childhood asthma, wheezing and respiratory infections. Jiang et al. contribute an important study on asthma severity in children with comorbid obesity in China. This reminds us that inflammation and immune modulation may be a common pathway linking asthma and obesity; both associated with disrupted microbiota.

With growing evidence of a plausible and impactful causal mechanism at play in patients and in experimental models, this issue focuses on the relevance of recent findings to population health. Dai et al. contribute important thinking on how such findings relate to health equity and cross-generational risk of chronic disease. Their article makes cogent arguments about how safeguarding the early-life microbiota may not only reduce atopic disease risk but also contribute to reducing intergenerational public health disparities.

Yang et al. contribute a regional study that characterises the burden that childhood asthma places on families through missed school days, missed work days, hospital visits and medical costs. Li et al. provide a modelling study that probes the impact of an observed 70% drop in infant antibiotic exposure on asthma burden in British Columbia, Canada. Very large drops in incident asthma cases, person-years with asthma and exacerbations have been realized, highlighting that asthma reduction should be considered in the value proposition of public health interventions such as antibiotic stewardship.

Mamun et al. address whether the observed effects of reducing exposure to antibiotics and promoting breastfeeding should have a significant effect at the population level. They observe that large declines in asthma seen in high income countries are in line with modelled predictions driven by lower exposure to antibiotics and improved uptake of breastfeeding. Falling asthma rates in British Columbia, Canada and in Germany have been linked to significant drops in antibiotic exposure (6, 7). Large drops in asthma have also been recorded in other countries where researchers are now looking for such an association (8).

Much remains to be done. Findings need to be replicated in additional cohorts and populations. Work is needed to quantify infant antibiotic exposure in resource-limited settings and identify opportunities to safely reduce unnecessary use. The complexities of competing risks are large. Antibiotic use for infants will be more important and more difficult to reduce in settings with higher sepsis rates. Approaches that have led to less antibiotic use in Canada, Germany or the UK may not be viable in other epidemiological contexts. Yet, observations outlined in this issue also remind us that atopic disease risk associated with antibiotics may be mitigated where breastfeeding or donor milk are available. A detailed understanding of beneficial bacteria and their function may also lead to better design of probiotics, prebiotics and synbiotics for the benefit of infants who need antibiotics and cannot be fed human milk.

This series provides a comprehensive introduction to experimental, population, cohort and modelling studies in this key area. When considered in aggregate, the evidence points to a significant potential to reduce the burden of atopic disease where it is possible to reduce unnecessary antibiotic use and encourage

human milk feeding for infants, alongside existing evidence of the benefit of improved air quality. Antimicrobial resistance and optimal infant nutrition are already sound reasons for such actions, but it now appears increasingly likely that they may also have a sizable impact on the scale of atopic disease pandemics, morbidity and costs.

Author contributions

DP: Writing – original draft, Writing – review & editing. ST: Writing – review & editing. MA: Writing – review & editing. PZ: Writing – review & editing. AD: Writing – review & editing. HL: Writing – review & editing.

Acknowledgments

The authors acknowledge an unrestricted grant in aid from the Pacific Public Health Foundation to offset publishing costs.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

The handling editor ASG declared a shared affiliation with the author MA at the time of review.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

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frontiers | Frontiers in Public Health

TYPE Systematic Review
PUBLISHED 24 May 2024
DOI 10.3389/fpubh.2024.1402908

OPEN ACCESS

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RECEIVED 18 March 2024
ACCEPTED 22 April 2024
PUBLISHED 24 May 2024

CITATION
Keleb A, Daba C, Asmare L, Bayou FD, Arefaynie M, Mohammed A, Tareke AA, Kebede N, Tsega Y, Endawkie A, Kebede SD, Abera KM, Abeje ET and Enyew EB (2024) The association between children's exposure to pesticides and asthma, wheezing, and lower respiratory tract infections. A systematic review and meta-analysis.
Front. Public Health 12:1402908.
doi: 10.3389/fpubh.2024.1402908

The association between children's exposure to pesticides and asthma, wheezing, and lower respiratory tract infections. A systematic review and meta-analysis

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Background: Exposure to pesticides is a global public health problem, especially for children. Its association with chronic respiratory disease among children has attracted considerable attention, but the existing evidence remains inconclusive and cannot be certain. Therefore, this systematic review and meta-analysis aim to determine the global pooled effect size of association with pesticide exposure and asthma, wheezing, and respiratory tract infections among children.

Methods: A comprehensive search was conducted for relevant literature from electronic databases, including PubMed, Google Scholar, Hinari, Semantic Scholar, and Science Direct. Studies that provided effect size on the association between pesticide exposure and childhood asthma, wheezing, and respiratory tract infections in children were included. The articles were screened, data was extracted, and the quality of each study was assessed with four independent reviewers. Random effects models for significant heterogeneity and fixed effect models for homogeneous studies were conducted to estimate pooled effect sizes with 95% confidence intervals using Comprehensive Meta-Analysis version 3.3.070 and MetaXL version 2. Funnel plot and Higgins I^2 statistics were used to determine the heterogeneity of the included studies. Subgroup analyses were computed based on the types of pesticide exposure, study design, sample size category, and outcome assessment technique.

Result: A total of 38 articles with 118,303 children less than 18 years of age were included in this meta-analysis. Pesticide exposure among children increased the risk of asthma by 24%; (OR = 1.24, 95% CI: 1.14–1.35) with extreme heterogeneity

(I^2 = 81%, p < 0.001). Exposure to pesticides increased the odds of developing wheezing among children by 34% (OR = 1.34, 95% CI: 1.14–1.57), with high heterogeneity (I^2 = 79%, p < 0.001) and also increased the risk of developing lower respiratory tract infection by 79% (OR = 1.79, 95% CI: 1.45–2.21) with nonsignificant low heterogeneity (I^2 = 30%, p-value = 0.18).

Conclusion: This meta-analysis provided valuable evidence supporting the association between childhood asthma, wheezing, and lower respiratory tract infection with pesticide exposure. The findings would contribute to a better understanding of the estimate of the effect of pesticide exposure on respiratory health in children and inform evidence-based preventive strategies and public health interventions.

KEYWORDS

pesticide exposure, chronic respiratory diseases, asthma, respiratory tract infection, children, systematic review, meta-analysis

Introduction

In the 21st century, pesticide exposure continues to be a serious global public health concern, especially for children. Approximately 300,000 deaths per year are attributable to pesticide exposure, which affects about 3 million people globally (1). Data from poison control centers showed that 3.4% of pediatric deaths and 3.6% of adult deaths are attributable to pesticide poisoning, with 3.3% of unintentional poisoning deaths coming from all sources (2). It was indicated that children aged less than 19 years of age accounted for about 59% of all single-substance pesticide exposures, and 94% of all pesticide ingestions were done inadvertently. The associated costs of treating chronic illness are very substantial, especially in developing nations (1, 3).

There is a risk of accidental and occupational exposure to pesticide residues of many kinds, including pyrethroids, fungicides, organochlorine (OC), organophosphate (OP), (4), indoor metabolites, and other chemicals (5). Different studies indicated that mostly organophosphate pesticides can cause neurotoxicity, immune toxicity, genotoxicity, nephrotoxicity, hepatotoxicity, cardiotoxicity, and reproductive toxicity (6–8), while organochlorine and pyrethroid pesticides can cause both acute and chronic respiratory disease and allergic reactions (9–11).

Adult epidemiological research indicates that occupational and environmental exposure to pesticides is linked to a high incidence of respiratory illnesses and their symptoms (12), including asthma, wheezing, respiratory tract infections (13), and changes in lung function (10, 14, 15). According to the pooled prevalence from a meta-analysis of 56 publications, the ratio of forced expiratory volume in 1 s to forced vital capacity decreased as a result of exposure to organophosphate pesticides (16–19). This is also supported by three literature reviews that were recently published (9, 20, 21). However, little is known about the pooled effect size of exposure to pesticides and childhood chronic respiratory diseases, including asthma, wheezing, and other respiratory tract infections.

Exposure to pesticides has been associated with an increased risk of chronic respiratory diseases and symptoms in children, and they are particularly vulnerable to asthma, wheezing, and lower respiratory tract infections (20) due to their developing bodies, immune systems, and behaviors that may increase their exposure (22–25). Children are mostly exposed to pesticides through inhalation (compared to adults, children breathe more about their body weight) (10), consumption of food and drink that has a high pesticide residual content (17, 23), by skin contact or exposure while using pesticides at home to control pests (17), mothers' exposure to pesticides during pregnancy, and their hand-to-mouth habit (25, 26).

Studies have examined the association between pesticide exposure and chronic respiratory diseases during childhood, and the previous studies conducted have not reached a consensus. This systematic review and meta-analysis aim to consolidate and determine pooled evidence on the association between chronic respiratory diseases and symptoms including asthma, wheezing, respiratory tract infection, and pesticide exposure. Understanding and pooled evidence for this relationship is crucial in advocating for environmental and occupational health regulations and promoting preventive measures to minimize the impact on public health.

Methods

Reporting system and registration

We used primary studies that reported the association between single or multiple pesticide exposure and asthma and/or LRTI and/ or wheezing and/or among children from prenatal to 18 years of age worldwide. Fundamental principles of the Centre for Reviews and Dissemination's (CRD) guidance for undertaking reviews in healthcare and Preferred Reporting Items for Systematic Review and Meta-Analysis guideline (PRISMA) were employed to conduct this review. It was registered at the Protocols at the International

Prospective Register of Systematic Reviews (PROSPERO: CRD42020176826) available.[1]

Data sources, study period, searching strategies, and study selection

A comprehensive literature search was conducted in electronic databases, including PubMed, Google Scholar, Hinari, Semantic Scholar, and Science Direct. The search included studies published from the inception of the databases from 1991 up to December 2, 2023, and the studies included in the previous systematic review were reevaluated and incorporated in this meta-analysis.

An effort was made to get in touch with experts in the field to obtain further details about both published and unpublished research. In addition, relevant references in selected studies were examined thoroughly to find related studies that were not found in our search.

The MeSH and search filters were included in the search strategies (Pesticides OR Insecticides OR Organophosphate OR Carbamates) AND (Respiratory function OR Pulmonary function OR Respiratory symptoms OR Respiratory disease OR Respiratory Disorder OR Asthma OR Wheeze OR Bronchitis OR Dyspnea OR Cough OR Phlegm) AND Children (27). In addition to the above keywords, synonyms, abbreviated symbols, and other free keywords were used. Only full-text articles in the English language were considered for review, the reference lists were also manually checked, and similar articles feature of a database was used. The search was performed up to December 2, 2023, by four authors independently (AK, CD, YT, and ET).

Every included and excluded studies were screened using EndNote 20 and the Rayyan automation tool. Screening by title and abstract was conducted independently, followed by screening by the full texts of the included studies by four authors. Disagreement was solved by consensus, and the selection process was recorded in sufficient detail to complete a PRISMA 2020 flow diagram.

Inclusion and exclusion criteria

In this review, cohort, case–control, or cross-sectional studies conducted (without restrictions to study period and sample size, study setting, and published and unpublished) on the association between pesticide exposure and chronic respiratory diseases, including asthma, lower respiratory tract infections (LRTI), and wheezing, among children less than 18 years of age. Observational studies conducted among children exposed to pesticides, insecticides, organophosphate, or carbamates and their derivatives were eligible for this review and compared with children who were not exposed with more or less exposed to pesticides, insecticides, organophosphate, carbamates, or their derivatives.

Children exposed to pesticides from agricultural sources, including parental and antenatal exposure, through air, via contaminated food, were also included, but studies carried out on groups other than children were excluded. Included outcomes were effect size reported on the association between asthma, wheezing, lower respiratory tract infection, and pesticide exposure among children less than 18 years of age. However, qualitative studies, irretrievable studies, editorial letters, studies with poor methodological quality, and studies that did not report the outcome of interest were excluded from the meta-analysis.

Outcome assessment

The primary outcome of the study was to estimate the pooled effect size of the association between pesticide exposure and asthma, and LRTI and wheezing in the form of odds ratio.

Data extraction quality assessment

After all, articles were exported into the EndNote 20 version and the Rayyan automation tool to remove the duplicated articles. The remaining data were extracted using a standardized form (initially piloted on two included studies) with characteristics of studies, outcomes, and risk of bias on Microsoft Excel 2016. Cohort, case–control, and cross-sectional studies using the author involved in the study, year, country, study design, sample size, type of pesticide exposed, exposure metrics, exposure assessment method, timing of outcome measurement, outcome assessment, and children with chronic respiratory disease (asthma and lower respiratory tract infections)/chronic respiratory symptoms (wheezing) associated with pesticide exposure were performed by five authors (AK, CD, YT, ET, and EB). After five reviewers (LA, AE, FD, MA, and AM) screened the relevant articles for eligibility, the quality of each article was evaluated using the Joana Brigg Institute (JBI) critical appraisal checklist (28). The four writers (AK, CD, AAT, and NK) evaluated the risk of bias for each study separately, and their scores were expressed on a 100% scale. A quality score of greater than 50% was used to include articles for further qualitative and quantitative analysis (28, 29). In the case of any discrepancies encountered during the quality assessment, the mean score was computed from the evaluations of all reviewers to address and resolve any differences.

Data analysis and synthesis

All types of analysis were computed using Comprehensive Meta-Analysis (CMA) version 3.3.070 and MetaXL version 2. The pooled effect sizes of the association between asthma, wheezing, and pesticide exposure were calculated using the random effects models.

The I^2 statistic was used to measure heterogeneity among the included studies and I^2 values less than 50% were considered homogeneous, and I^2 values greater than or equal to 50% were

1 https://www.crd.york.ac.uk/Prospero

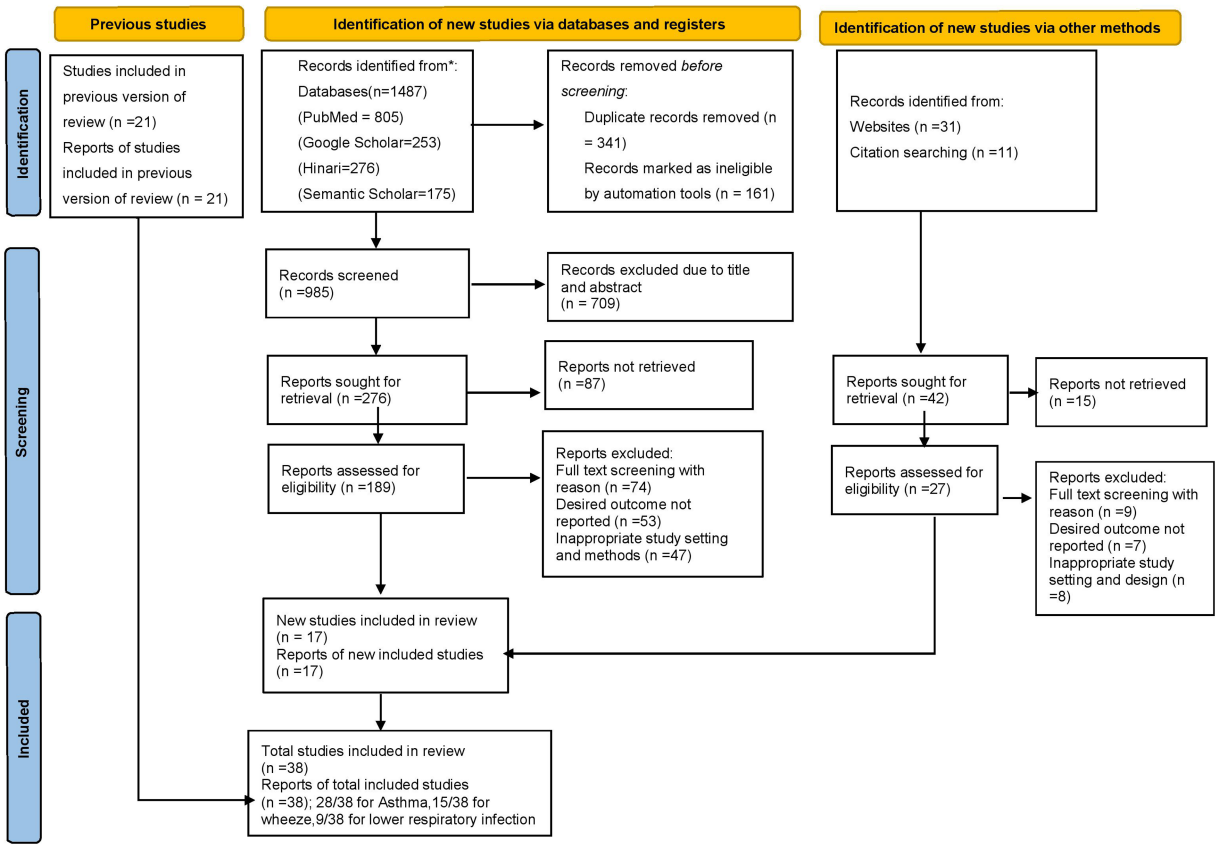

FIGURE 1
PRISMA flow diagram of the included studies for the systematic review and meta-analysis of respiratory diseases, 2023.

considered as of high heterogeneity. Begg's funnel plots and Eggers test were employed to assess publication bias/small studies effect. A 95% Confidence Interval (CI) and *p*-value of less than 0.05 were considered significant for the association, absence of publication bias, and heterogeneity.

Subgroup analyses were conducted on different factors to identify sources of heterogeneity, including sample size (large vs. small), types of study design (case–control, cohort, cross-sectional), types of pesticide exposure (multiple vs. single), and types of outcome measurement (biomarker, doctor diagnosed, and self-reported) for asthma. Furthermore, to resolve heterogeneity further, sensitivity analysis was performed by removing one study in each scenario.

Results

Study selection and characteristics of the included studies

Based on the search study stated above, 1,487 studies from databases, 31 from websites, and 11 from citations were identified. A total of 502 studies from the database were discarded due to duplication. About 341 discarded studies were excluded via EndNote 20, and the remaining 161 studies were excluded using the Rayyan automation tool. Title and abstract parts of the remaining 985 studies were reviewed, of which 709 studies were excluded due to irrelevance. Out of the 276 studies that were sought to be retrieved, 87 could not be retrieved, and 189 were eligible for full-text screening. Finally, 15 studies from the new database, 2 studies from the website and citation, and 21 articles screened and reevaluated from previous reviews were eligible and included in the study. PRISMA flow chart related to the search process is shown in Figure 1.

Characteristics of the included studies (qualitative review)

A total of 38 articles (23, 27, 30–66) were included to determine the association between exposure to pesticides, chronic respiratory diseases, and symptoms including asthma, wheezing, and respiratory tract infections. In this meta-analysis, a total 118,303 of children as study subjects were included. In this meta-analysis, 13 studies were carried out in United states of America (27, 31, 33, 35–38, 49–51, 53, 56, 66), four from Spain (34, 59–61), two from China (46, 63), two in Canada (43), two in South Africa (30, 39), three in Lebanon (44), two from Costa Rica (40, 47), one each from six countries (Italy, Germany, Romania, Netherland, one combined

study from Greenland and Ukraine, and one combined study from North Europe and Australia) (32, 41, 42, 45, 48, 57), and one from England (62). Characteristics of all the included studies are summarized in Table 1.

The association of pesticide exposure and childhood asthma

A total of 28 studies (27, 30, 32–36, 38, 41–43, 46, 48, 49, 52, 54–58, 60, 63, 64) were included in the random effect model meta-analysis to examine the association between childhood asthma and pesticide exposure. Pesticide exposure had a statistically significant association between childhood asthma and pesticide exposure with pooled effect size, (OR = 1.24, 95% CI = 1.14–1.35, p-value <0.001) with significant extreme heterogeneity ($I^2 = 81\%$, $p < 0.001$) among included studies (Figure 2).

The association of pesticide exposure and childhood wheeze

Fifteen studies (30, 34, 37, 39, 40, 43–45, 53, 55, 57, 60, 61, 66) were included in the meta-analysis for the association between pesticide exposure and wheezing among children. The result from the random effect model indicated that pesticide exposure had a statistically significant association with the occurrence of childhood wheezing (OR = 1.34, 95% CI = 1.14–1.57, p-value <0.001) and

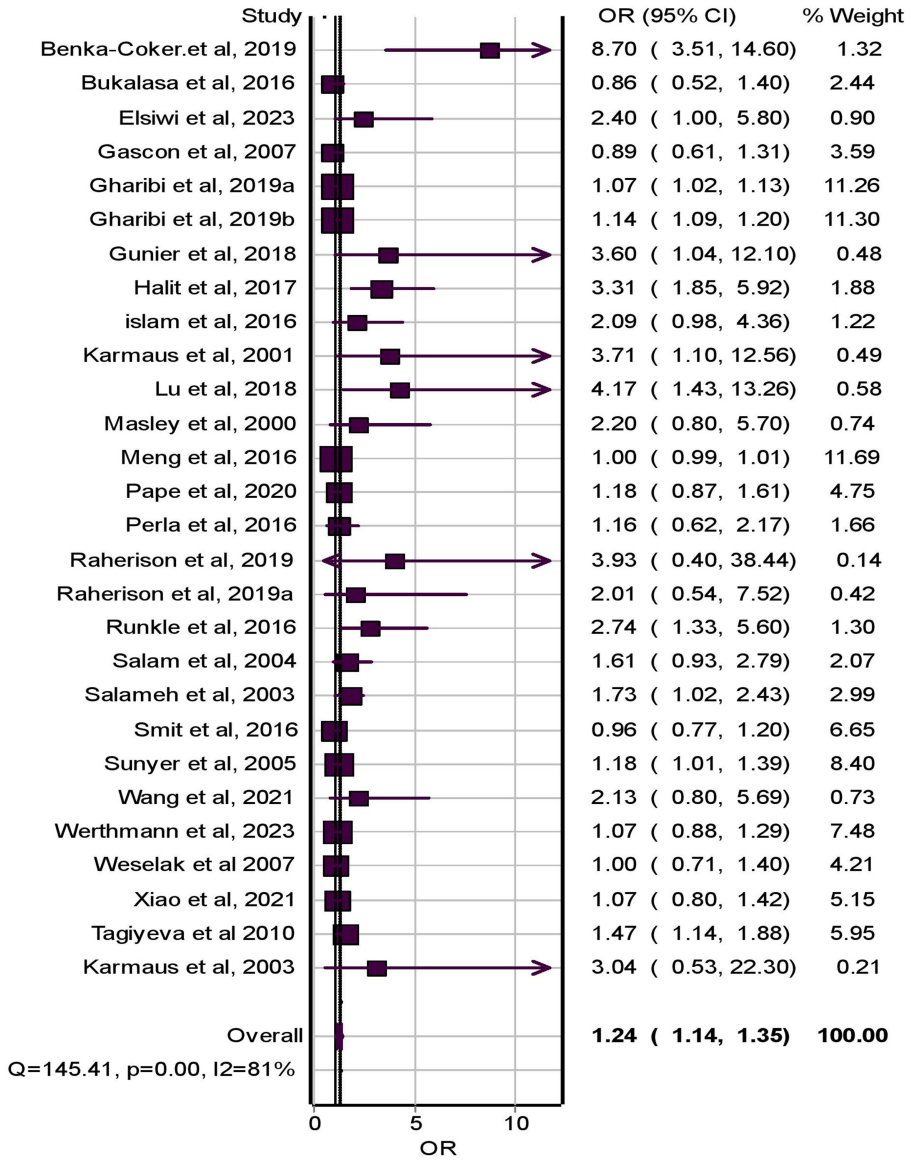

FIGURE 2
Forest plot of odds ratios for the association of pesticide exposure and childhood asthma, 2023.

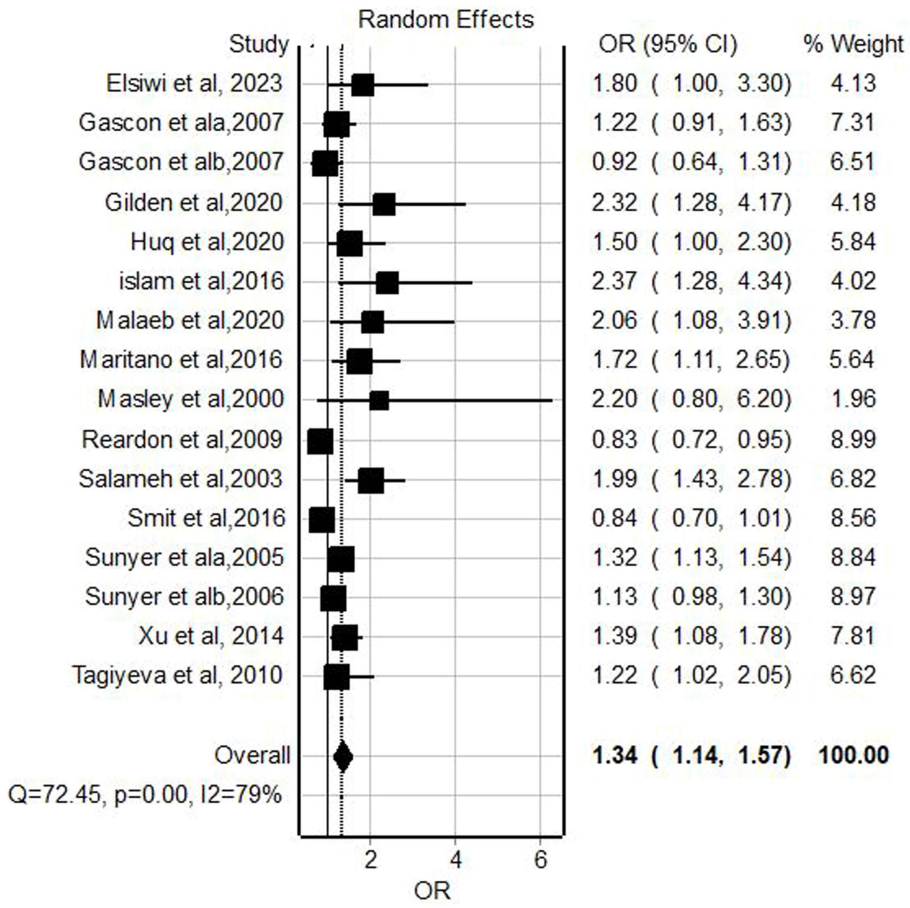

FIGURE 3
Forest plot of odds ratios for the association of pesticide exposure and childhood wheeze, 2023.

significant extreme heterogeneity ($I^2 = 79\%$, $p < 0.001$) within included studies (Figure 3).

The association of pesticide exposure and lower respiratory tract infections

Meta-analysis of nine relevant studies (34, 40, 43, 47, 55, 58, 59, 63, 64) using fixed effect model showed a significant association between lower respiratory tract infections and pesticide exposure with a pooled odd ratio of 1.79 (95% CI = 1.45–2.21, p-value <0.001) with nonsignificant low heterogeneity ($I^2 = 30\%$, p-value = 0.18; Figure 4).

Publication bias

Publication bias occurs when research with significant results is more likely to be published than those with nonsignificant results. This bias may cause effect sizes to be overestimated and an inflated perception of the strength of the association. To mitigate the possibility of selective publication, a thorough literature search utilizing multiple databases was carried out to reduce publication bias. Egger's test and Begg's funnel plots were used to evaluate any potential publication bias quantitatively. For wheezing and asthma, publication bias was found (p value <0.05). To estimate the effect size by imputing or "filling in" potentially missing data, trim and fill analysis was carried out on the left using a fixed model (adjusted values from 12 trimmed studies with a point estimate of 1.01, with 95% CI = 1.00–1.02 for asthma and 6 trimmed studies with a point estimate of 1.16. with 95% CI: 1.08–1.24 for wheeze). However, publication bias was not detected in the case of lower respiratory infection (p-value = 0.403) during meta-analysis (Figure 5).

Subgroup analyses for asthma

Statistically significant association (OR = 1.32, 95% CI, 1.15–1.51, $p < 0.001$) with extreme heterogeneity ($I^2 = 85.6\%$, $p < 0.001$) was detected among studies with small sample sizes (<1,000). The effect sizes between pesticide exposure and childhood asthma vary moderately for both cohorts, (OR = 1.19, 95% CI: 1.02–1.39, $p = 0.025$, with $I^2 = 75.9\%$, $p < 0.001$) and cross-sectional studies (OR = 1.35, 95% CI: 1.14–1.59, $p = 0.001$, with $I^2 = 65.2\%$, $p < 0.001$) in contrast to the nonsignificant association for case–control studies (Supplementary Table S1).

Studies investigating multiple pesticide exposures reported a significant association of 1.27 (95% CI: 1.10–1.94, $p = 0.002$) with moderate significant heterogeneity ($I^2 = 58.8\%$, $p < 0.000$), whereas

TABLE 1 Characteristics of all the included studies based on the pesticide exposure and respiratory health outcomes, 2023.

Author	Year	Country	Study design	Study sample	Pesticide addressed	Exposure metrics	Exposure assessment method	Timing of outcome measurement	Outcomes assessment method	Health effects and its association
Benka-Coker. et al.	2019	USA	Cohort	16	OP	agriculture	urine samples for DAP (summative measures)	Not clear	Biomarkers (Urinary LTE)	Asthma 8.7(95%CI:3.512,14.600)
Bukalasa et al.	2016	Netherlands	Cohort	1,470	Multiple exposure	agriculture	Questionnaire interview	0–14 years old	Self-reported	Asthma OR = 0.860, 95%CI:0.520, 1.400 with 1KM distance Respiratory symptoms OR = 0.860, 95%CI:0.520, 1.400 with 1KM distance
Elsiwi et al.	2023	South Africa	Cohort	620	Pyrethroid	environmental	maternal urine 3PBA	up to 5 years	Doctor diagnosed	Asthma, (OR = 2.400, 95%CI:1.000, 5.800) Wheeze, OR = 1.80 95%CI: 1.00, 3.30
Famid et al.	2020	South Africa	Cohort	652	Organochlorine	Prenatal	Cord blood DDT	Up to 3.5 years	Doctor diagnosed	Wheeze, (OR = 1.500, 95%CI:1.000, 2.300)
Gascon et al.	2007	Spain	cohort	405	DDE/OC	environmental	cord blood DDE+ immune biomarker	birth to 14 years old	doctor diagnosed	Asthma 10 years, RR = 1.03 (95%CI: 0.71, 1.50) 14 years, RR = 0.89 (95%CI: 0.61, 1.31) Wheeze 10 years, RR = 1.22 (95%CI: 0.91, 1.63) 14 years, RR = 0.92 (95%CI: 0.64, 1.31) LRTI 10 years, RR = 1.27 (95%CI: 0.86, 1.86)
Gharibi et al. a	2019a	USA	cross sectional	4,262	methyl bromide	environmental	questionnaire interview	2–18 years old	doctor diagnosed	Asthma OR = 1.02, 95% CI: 0.99, 1.05, for 2–5 years OR = 1.07, 95% CI: 1.02, 1.12 for 6–18 years
Gharibi et al. b	2019b	USA	cross sectional	1,331	1,3-dichloropropene	environmental	questionnaire interview	2–18 years old	doctor diagnosed	Asthma (OR = 1.06, 95%CI: 1.02, 1.11 OR = 1.14, 95%CI: 1.07, 1.19)
Gilden et al.	2020	USA	Cohort	390	OP and pyrethroid	Prenatal	urinary DAP and 3PBA	Up to 8 years old	interview and spirometry	Wheeze OR = 2.32, 95%CI: 1.28, 4.17
Gunjer et al.	2018	USA	Cohort	294	multiple pesticides	Agriculture	questionnaire interview	prenatal to 7 years	Interview and Spirometry	Asthma OR = 3.60, 95%CI: 1.04, 12.10
Halit et al.	2017	Lebanon	case control	1,503	multiple pesticides	environmental	questionnaire interview	3–16 years old	self-reported	Asthma OR = 2.709, 95%CI: 1.219, 6.020 LRTI OR = 2.71 (95%CI: 1.22, 6.02)
Huq et al.	2020	South Africa	Cohort	658	multiple	Prenatal	Questionnaire	3.5 years	Doctor diagnosis	Wheeze OR = 1.500 (95%CI: 1.000, 2.300)

(Continued)

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TABLE 1 (Continued)

Author	Year	Country	Study design	Study sample	Pesticide addressed	Exposure metrics	Exposure assessment method	Timing of outcome measurement	Outcomes assessment method	Health effects and its association
Islam et al.	2016	Costa Rica	cross sectional	303	Pyrethroid	Agriculture	questionnaire interview	up to 5 year	Doctor diagnosed	Asthma OR = 2.090, 95%CI: 0.980, 4.360 Wheeze OR = 2.37(95%CI: 1.28, 4.34) LRTI OR = 2.78 (95%CI: 0.41, 8.04)
Kuramaus et al.	2001	Germany	cross sectional	343	OC/DDE	Agriculture	cord blood DDE	3–7 years old	Self-reported	Asthma OR = 3.710, 95%CI: 1.100, 12.560,
Kuramaus et al.	2003	Germany	cross sectional	338	OC/DDE	Agriculture	cord blood DDE	7–10 years old	Self-reported	Asthma OR = 3.040, 95%CI: 0.530, 22.300,
Lu et al.	2018	Romania	cross sectional	280	multiple pesticides	environmental	questionnaire interview	6–11 years old	self-reported	Multi-pollutant controlled to CO2 vs. asthma OR = 4.17 (5%CI:1.430, 13.260)
Malaeb et al.	2020	Lebanon	cross sectional	1,203	multiple pesticides	environmental	questionnaire interview	4–7 years	self-reported	Wheeze OR = 2.06(95%CI: 1.08, 3.91)
Maritano et al.	2016	Italy	case control	5,346	multiple pesticides	environmental	questionnaire interview	6–18 months	self-reported	Wheeze OR = 1.72(95%CI: 1.11, 2.65)
Masley et al.	2000	Canada	cross sectional	393	multiple pesticides	Agriculture	questionnaire interview	Up to 17 years old	self-reported	Asthma OR = 2.200, 95%CI: 0.800, 5.700 Wheeze OR = 2.20(95%CI: 0.80,6.02) Bronchitis/RTI OR = 2.80 (95%CI: 1.60, 4.80)
Meng et al.	2016	China	case control	620 case/218 control	OC	environmental	questionnaire interview	3–6 years	doctor diagnosed	Severe Asthma OR = 1.000, 95%CI:0.99, 1.002,
Mora et al.	2020	Costa Rica	Cohort	355	Fungicides	Prenatal	Seven Urinary metabolites	1st trimester to 19 months of postpartum	Biomarker	Wheezing OR = 0.69 (95%CI: 0.37, 1.28) LRTI OR = 1.500, 95%CI:0.70, 3.19,
Pape et al.	2020	North Europe & Australia	Cohort	2,766	Multiple exposure b	prenatal and environmental	Questionnaire interview	0–15 years old	Self-reported	Asthma OR = 1.180, 95%CI:0.870, 1.610
Perla et al.	2016	USA	cross sectional	10,077	OP and DDT	environmental	blood and urine DAP test	Up to 16 years	Biomarker	Ever Asthma >75th percentile RR = 1.160, 95%CI: 0.620, 2.170,

(Continued)

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TABLE 1 (Continued)

Author	Year	Country	Study design	Study sample	Pesticide addressed	Exposure metrics	Exposure assessment method	Timing of outcome measurement	Outcomes assessment method	Health effects and its association
Raanan et al.	2015	USA	Cohort	342	OP/DAPs	Agriculture	Maternal interview& urinary DAPs	prenatal up to 7 years	Self-reported respiratory symptoms	Respiratory symptoms OR = 2.530, 95%CI:1.320, 4.86, from children
Raanan et al.	2017	USA	Cohort	347	Elemental sulfur	agriculture	Pesticide use report	Short term exposure	Self-report	Respiratory symptoms/wheeze (OR = 2:09; 95% CI: 1.27; 3.46, p = 0.004) with 1KM distance
Raherison et al.	2019	France	cross sectional	281	multiple pesticides	environmental	urine ETU and air monitoring	not clear	Biomarker and clinical	Pesticide in air vs. Asthma OR: 3.930, 95% 0.400–38.440 Urinary ETU vs. Asthma OR = 2.010, 95%CI: 0.540, 7.520
Reardon et al.	2009	USA	Cohort	652	OP + Pyrethroids	Agriculture	questionnaire interview	prenatal to 12 months	Biomarkers	Wheeze (OR = 0.83,95%CI: 0.72–0.95)
Salam et al.	2004	USA	case control	4,000	multiple pesticides	environmental	questionnaire interview	prenatal to 12 months	doctor diagnosed	Asthma (OR = 1.61; 95% CI, 0.930–2.790)
Salameh et al.	2003	Lebanon	cross sectional	3,291	multiple pesticides	environmental	questionnaire & residential exposure score	3 years −16 years	self-reported	Asthma (OR = 1.73; 95%CI: 1.02–2.97), Respiratory disease/RTI (OR = 1.71; 95%CI: 1.20–2.43), Ever wheezing (OR = 1.99; 95%CI: 1.43–2.78)
Smit et al.	2016	Greenland & Ukrain	Cohort	1,024	OC/PCB 153	environmental	blood sample PCB	5–9 years	doctor diagnosed	Asthma, (OR = 0.960, 95%CI:0.770, 1.200) Wheeze,(OR = 0.840, 95%CI:0.700, 1.010)
Sunyer et al.	2005	Spain	Cohort	468	OC/DDE	Agriculture	cord blood DDE	at age 4 year	Doctor diagnosed	Wheeze OR = 1.32, 95%CI:1.130, 1.540
Sunyer et al.	2006	Spain	Cohort	402	OC/DDE	Agriculture	cord blood DDE	at age 6.5 year	Doctor diagnosed	asthma (OR = 1.180, 95%CI:1.01, 1.39) Wheeze,(OR = 1.130, 95%CI:0.980, 1.300)
Sunyer et al.	2010	Spain	Cohort	584	OC/DDE	Prenatal	Maternal serum	6–14 months	Doctor diagnosis	RTI/LRTI (OR = 2.40, 95%CI:1.19, 4.83)
Tagiyeva et al. 2010	2010	England	Cohort	13,971	Biocides and fungicides	Maternal postnatal exposure	Maternal interview	0–8.5 years	Doctor diagnosis	Asthma (OR = 1.470, 95%CI: 1.100, 1.880)
Wang et al.	2021	China	cross sectional	627	Insecticide	Residential	survey using questionnaire	school children	interview and spirometry	Asthma OR = 2.128 (95%CI: 0.796, 5.689) Bronchitis/RTI OR = 2.05 (95%CI: 1.38, 3.05)

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TABLE 1 (Continued)

Author	Year	Country	Study design	Study sample	Pesticide addressed	Exposure metrics	Exposure assessment method	Timing of outcome measurement	Outcomes assessment method	Health effects and its association
Werthmann et al.	2023	USA	Cohort	162	multiple pesticides	environmental	Urinary pesticide metabolite (3,PBA)	7–12 years	doctor diagnosed	Asthma (OR = 1.070, 95%CI: 0.880, 1.290)
Weselak et al.	2007	Canada	Cohort	3,405	multiple pesticides	Agriculture	questionnaire interview	Prenatal	self-reported	Any pesticide vs. Asthma (OR = 1.000, 95%CI: 0.710, 1.400) Bronchitis/RTI OR = 1.21 (95%CI: 0.77, 1.90)
Xiao et al.	2021	USA	cross sectional	41,423	multiple pesticides	household	survey using questionnaire	up to 17 year	self- reported	Asthma OR = 1.070 (95%CI: 0.800, 1.420)
Xu et al.	2014	USA	cross sectional	14,065	multiple pesticides	Residential	questionnaire interview	1–17 years old	self-reported	Wheeze OR = 1.390 (95%CI: 1.080, 1.780)

studies focused on single pesticide exposures found a smaller but still significant effect size of 1.16 (95% CI: 1.05–1.29, $p = 0.004$) with higher significant heterogeneity ($I^2 = 85.6\%$, $p < 0.001$) among the included studies (Table 2).

Sensitivity analysis

A sensitivity analysis was carried out to assess the robustness of the meta-analysis findings. The total effect size estimates were consistent and statistically significant, even after individual trials were systematically removed to evaluate their impact. Removing studies with a high potential for bias had no discernible impact on the findings. The pooled effect size estimate held true after accounting for publication bias, which was evaluated. However, it is imperative to consider data limitations and potential sources of heterogeneity when interpreting the results.

Discussion

Pesticides, such as fungicides, insecticides, and herbicides, have been widely used since the 1950s to increase crop yields (67). Exposure to these substances raised knowledge of the risks associated with respiratory diseases and/or symptoms (14, 16, 19, 68). This review demonstrates that numerous epidemiological studies showed an association between children's exposure to pesticides (from household, prenatal, postnatal, caregiver agricultural activities, residential, and environmental sources) and an increased risk of developing respiratory tract infections, asthma, and wheezing.

Because of the nature of the study design or the use of retrospective questionnaires, the measurement of pesticide exposure is frequently restricted in particular studies. However, 16 studies assessed the levels of pesticide metabolites in blood and urine, which is a more trustworthy estimate than utilizing a questionnaire during an interview.

The objective of this systematic review and meta-analysis aimed to determine the pooled effect size between prenatal, occupational, and environmental pesticide exposure and chronic respiratory disease/symptoms including asthma, lower respiratory tract infection, and wheezing globally. A significant association was found between pesticide exposure and asthma among children in this review aligns with previous systematic reviews and meta-analyses that have reported positive associations between pesticide exposure and asthma (18–21, 69). These concordant results across different meta-analyses strengthen the evidence for the association and underscore its significance in the field (22, 23, 70), and two recent studies with new perspectives have investigated the association between exposure to pesticides in indoor dust and respiratory outcomes including asthma and wheezing (5, 71). However, Mthethwa et al. (72) found that inconsistent patterns of increased risk of asthma outcomes with increasing organophosphate concentrations among school children, and Gunier et al. (38) also reported a negative association between pesticide exposure and childhood asthma.

The subgroup analysis of childhood asthma within this study revealed significant variability among the included studies. The

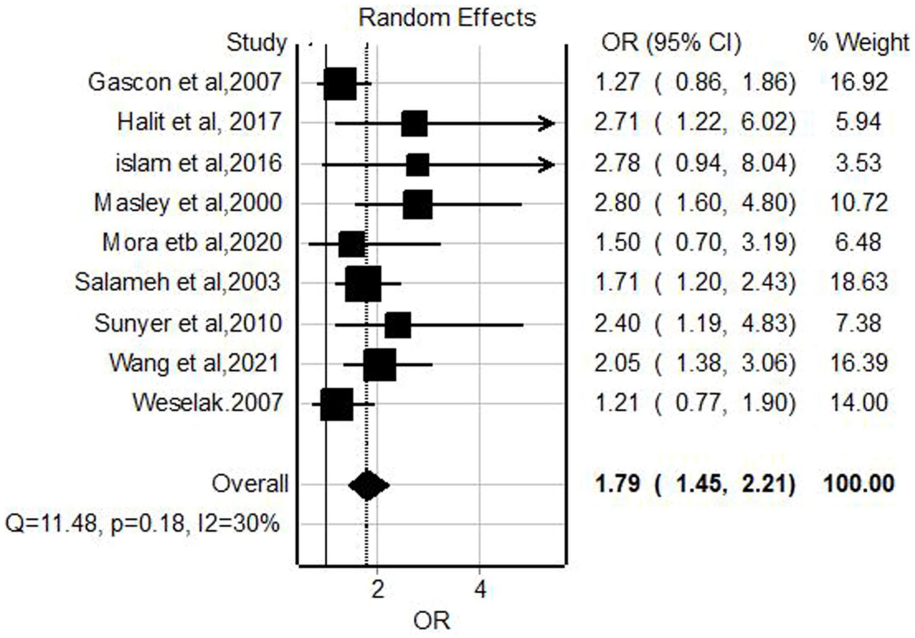

FIGURE 4
Forest plot of odds ratios for the association pesticide exposure and lower respiratory infections among children, 2023.

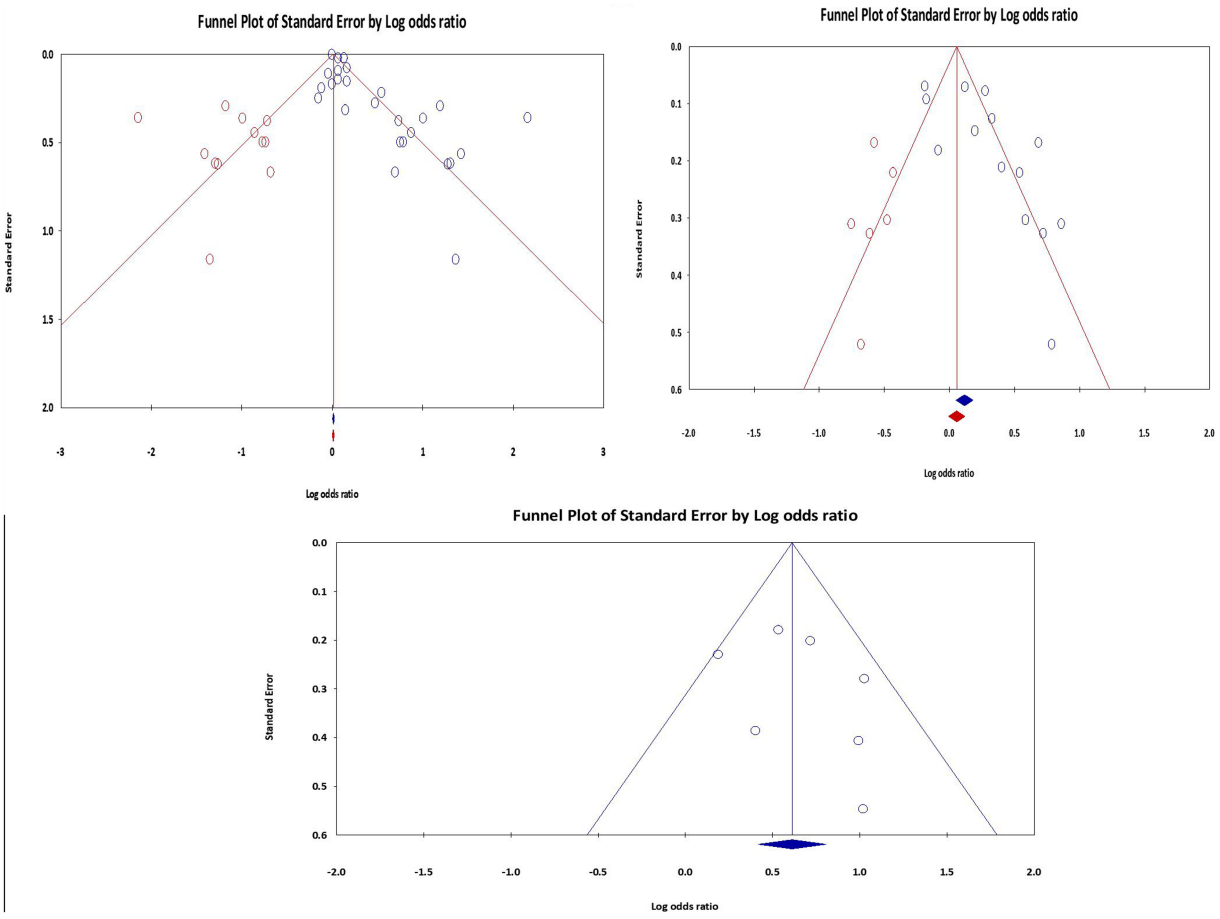

FIGURE 5
Funnel plot of association of pesticide exposure and asthma, wheeze and LRTI, 2023.

TABLE 2 Subgroup analysis of the pooled effect size and heterogeneity of asthma among children exposed to pesticide globally, 2023.

Variable	Category	No of studies	Effect size and significance		Heterogeneity	
			OR (95%CI)	*p*-value	I^2	*p*-value
Sample size	Large(≥1,000)	11	1.14(0.99–1.32)	0.077	36.7%	0.115
	Small(<1,000)	17	1.32(1.15–1.51)	<0.000	85.6%	<0.000
Study design	Case control	3	1.21(0.92–1.60)	0.174	77.4%	0.012
	Cohort	13	1.19(1.02–1.39)	0.025	75.9%	<0.000
	Cross sectional	12	1.35(1.14–1.59)	0.001	65.2%	0.001
Types of exposure	Multiple pesticide	13	1.27(1.09–1.47)	0.002	58.8%	0.005
	Single pesticide	15	1.16 (1.05–1.29)	0.004	85.6%	<0.000
Outcome measurement	Biomarkers	7	1.19(0.96–1.48)	0.110	54.5%	0.040
	Doctor diagnosed	13	1.18(1.05–1.34)	0.004	87.9%	<0.000
	Self-reported	7	1.23(1.05–1.45)	0.011	39.7%	0.077

general possible reasons for this variation seen in the study might include differences in exposure assessment techniques (self-reporting, biomarkers, doctor diagnosed, or environmental monitoring), outcome ascertainment (diagnostic criteria and follow-up durations), populations' characteristics (demographics, geographic locations, occupational exposures, and underlying health conditions), and publication bias, as well as variations in study designs, such as cohort, case–control, or cross-sectional studies.

Significant heterogeneity might impact the interpretation and generalizability of meta-analytic results; hence subgroup analysis was computed very carefully on types of study design, sample size, level of exposure, and method of measurement to produce subgroup effect sizes that can be interpreted in the context of the observed variability.

A significant level of heterogeneity among studies characterized by small sample sizes may be because small sample sizes lead to less precise estimates and greater susceptibility to chance variations or biases. Additionally, differences in exposure assessment, outcome measurement, children's characteristics, and study design across these studies may contribute to the observed heterogeneity.

A significant heterogeneity across cohort and cross-sectional studies in this meta-analysis can be attributed to several factors, including temporal limitations in establishing causality, children's characteristics such as age distribution, genetic predisposition, and environmental factors, potential bias, and unmeasured confounding variables.

Cross-sectional studies may encounter limitations in establishing temporal relationships due to the simultaneous assessment of exposure and outcome, making it challenging to determine the direction of causality accurately. Despite significant differences across the studies, 13 birth cohort studies with fewer recall bias and better control of confounders strongly suggest a causal association between pesticide exposure and asthma in children.

Both multiple- and single-pesticide exposure studies showed significant heterogeneity within each exposure category, highlighting differences between studies focusing on multiple versus single exposures. This variation might be attributed to differences in the types and combinations of pesticides across regions, individual susceptibility influenced by genetics and lifestyle, as well as variations in study design and methodology. These factors underscore the complexity of understanding the association between pesticide exposure and asthma among children.

The current meta-analysis also grouped studies according to how they measured the outcome (asthma) and found substantial variability between doctor-diagnosed asthma and biomarker measurements. This difference may stem from differences in diagnostic accuracy and variability in the interpretation of diagnostic criteria. Biomarkers provide objective measures of asthma, but there may still be variability in their use and interpretation across studies. On the other hand, doctor-diagnosed asthma relies on subjective assessment, which can vary greatly depending on individual clinician judgment, diagnostic criteria, and healthcare settings. These differences enforce to underscore the importance of standardized diagnostic approaches in research.

On the other hand, studies utilizing self-reported measures of asthma demonstrated nonsignificant heterogeneity. This may be because, in comparison to biomarker or doctor-diagnosed methods, self-reported measures of asthma typically capture a wider range of symptoms and experiences directly from individuals, which may result in higher sensitivity for detecting associations. Additionally, self-reported measures are typically more accessible and less resource-intensive, potentially leading to larger sample sizes and increased statistical power. Furthermore, a lower level of heterogeneity indicates more consistency in the methods used across studies employing self-reported measures, such as standardized questionnaires or protocols. However, it is important to acknowledge that reliance solely on self-reporting may introduce bias due to misclassification or recall errors, which could impact the observed effect sizes.

These subgroup findings also emphasize the need for further investigation and targeted research in specific children's age categories and follow-up. Future studies on pesticide exposure and childhood asthma need to focus on specific age cohorts, use rigorous methodology, and account for different variances. However, the inclusion of a large number of studies appropriately accounts for the observed heterogeneity between studies, which increases the generalizability of the findings. This comprehensive approach also enhances our understanding of the association between pesticide exposure and childhood asthma and emphasizes targeted preventive measures and other public health interventions.

A significant association between wheeze and pesticide exposure of this finding aligns with the previous studies that have reported positive associations between pesticide exposure and wheezing among children (19, 20, 25, 37, 44, 73), particularly, in residential, agricultural, environmental, and prenatal exposure (23, 68, 73) and school (71). However, the included studies had statistically significant high heterogeneity, which was highly contrasted from study to study.

The variations in findings across different studies may be attributed to methodological differences, such as variations in study design, sample size, and age category of children or differences in exposure assessment methods including self-reporting or biomarker measurements. Furthermore, variations in study populations, including demographic characteristics and occupational backgrounds of mothers, caregivers, and fathers of children, may also influence the observed associations.

This meta-analysis also identified a significant positive association between pesticide exposure and an increased risk of lower respiratory tract infections with nonsignificant low heterogeneity. A significant association between pesticide exposure and lower respiratory tract infections from this finding is consistent with several previous studies that have reported positive associations between pesticide exposure and LRTI among children (19, 23, 40, 45, 47, 60). However, some studies have reported contrasted results (74) potentially due to methodological differences, variations in exposure assessment, and study populations. Finally, the included studies indicated non-significant heterogeneity that did not vary from study to study.

Limitation and strength of the study

We identified several limitations on the reporting of windows of susceptibility, timing, length, and surrogates of exposure assessment. The majority of studies relied on questionnaires and self-reported exposure, which can be affected by recall bias and exposure misclassification. However, this meta-analysis followed the updated preferred reporting items for systematic review and meta-analysis. In this meta-analysis, all cases including asthma, wheezing, and lower respiratory tract infection as a result of exposure to pesticides were appropriately assessed.

Conclusion

This meta-analysis showed that children who are exposed to pesticides are at an increased risk of developing chronic respiratory diseases and symptoms specifically asthma, RTI, and wheezing. This is particularly concerning as respiratory health problems could have long-term effects on a child's health and well-being. Parents and other caregivers who care for children must understand the possible dangers of pesticide exposure and take precautions to reduce their exposure to these dangerous substances.

This can involve utilizing natural pest management techniques, selecting organic products, and pushing for stronger laws governing the use of pesticides in agricultural operations. Policymakers must take a leading role in safeguarding children from pesticide exposure by implementing regulations and policies that prioritize the health and safety of our most vulnerable populations to ensure that they have the opportunity to grow up in a healthy and safe environment.

Future research on pesticide exposure and asthma, wheezing, and LRTI among children should focus on longitudinal studies with an accurate assessment of pesticide exposure to capture cumulative long-term effects. Novel methods have been used to investigate the combined health effects of multiple pesticide exposures. Stratified analyses can also elucidate susceptibility factors, while mechanistic studies and metabolomics are essential for uncovering the biological pathways of pesticide toxicity.

Evaluating the effectiveness of preventive measures through intervention studies is better to inform strategies to mitigate respiratory health risks associated with pesticide exposure among children. Moreover, international research protocols tailored to local specificities should be developed and validated to compare studies conducted in different settings and enhance our understanding of the complexities of pesticide exposure and respiratory health outcomes among children.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

AK: Conceptualization, Data curation, Formal analysis, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. CD: Conceptualization, Data curation, Formal analysis, Methodology, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. LA: Data curation, Investigation, Methodology, Software, Supervision, Writing – original draft, Writing – review & editing. FB: Conceptualization, Data curation, Methodology, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. MA: Conceptualization, Data curation, Formal Analysis, Methodology, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. AM: Conceptualization, Data curation, Formal analysis, Methodology, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. AT: Conceptualization, Data curation, Methodology, Software, Supervision, Visualization, Writing – original draft, Writing – review & editing. NK: Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. YT: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. AE: Conceptualization, Data curation, Formal analysis, Methodology, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. SK: Writing – original draft, Writing – review & editing. KA: Conceptualization, Data curation, Methodology, Resources, Software, Supervision, Validation, Writing – original draft, Writing – review & editing. EE: Conceptualization, Data curation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. EB: Conceptualization, Data curation,

Formal analysis, Methodology, Software, Supervision, Validation, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1402908/full#supplementary-material

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 05 June 2024
DOI 10.3389/fpubh.2024.1368519

OPEN ACCESS

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RECEIVED 26 January 2024
ACCEPTED 23 May 2024
PUBLISHED 05 June 2024

CITATION
Yang F, Zhou J, Xiao H, Wu X, Cui Y, Huang H, Zheng S and Li H (2024) Caregiver burden among parents of school-age children with asthma: a cross-sectional study.
Front. Public Health 12:1368519.
doi: 10.3389/fpubh.2024.1368519

Caregiver burden among parents of school-age children with asthma: a cross-sectional study

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Objective: To investigate the caregiver burden of parents of school-age children with asthma and analyze the factors influencing their caregiver burden.

Methods: A convenience sampling method was used to select 366 parents of school-age children with asthma who visited the outpatient departments of three tertiary hospitals in Sichuan Province, China, from January 2021 to July 2021. A general information questionnaire and the Caregiver Burden Inventory (CBI) were used to assess the current caregiver burden and analyze the influencing factors.

Results: The caregiver burden score of parents of school-age children with asthma was 27 (17, 39), with 40.43% of parents experiencing moderate to high levels of burden. Detailed results of univariate analysis showed that there were significant differences in caregiver burden scores based on parents' gender, highest education level, number of children, occupation, family history of asthma, monthly family income, annual medical expenses for the child, child's gender, whether the child had undergone lung function tests, number of emergency visits due to asthma exacerbation in the past 3 months, and whether the child had missed school due to asthma exacerbation in the past 3 months ($p < 0.1$). Detailed results of multivariate analysis showed that parents' gender, occupation, family history of asthma, monthly family income, annual medical expenses for the child, number of emergency visits due to asthma exacerbation in the past 3 months, and whether the child had missed school due to asthma exacerbation in the past 3 months were independent risk factors for caregiver burden in parents of school-age children with asthma ($p < 0.05$).

Conclusion: Parents of school-age children with asthma experience a certain level of caregiver burden, with over one-third of parents experiencing moderate to high levels of burden. Being a mother, being a worker, having no family history of asthma, having low monthly family income, having high annual medical expenses for the child, having frequent emergency visits due to asthma exacerbation in the past 3months, and having missed school due to asthma exacerbation in the past 3months are independent risk factors for caregiver burden in parents of school-age children with asthma, healthcare providers should develop feasible coping strategies, such as paying attention to caregivers' psychological condition to reduce the burden of caring for parents of school-age children with asthma. The entire society should also make efforts in improving social support and strengthening healthcare coverage in order to achieve the aforementioned goals.

KEYWORDS

school-age, asthma, parents, caregivers, caregiver burden

1 Introduction

Asthma is one of the most common chronic respiratory disease worldwide (1), affecting over 300 million people globally (2). The prevalence of asthma is approximately 5.00–10.00% in adults and 20.00% in children (3), with school-age children (6–14 years old) being a high-risk group for asthma (4). Numerous studies have shown that asthma has various and significant impacts on the health, learning, and social interactions of affected children (5, 6), as well as on the daily life, physical and mental health of their primary caregivers and families (7, 8).

Caregiver burden refers to the negative physiological, psychological, and economic stimuli and pressures experienced by caregivers during the caregiving process (9, 10). As the primary caregivers of school-age children with asthma (11), parents bear a series of burdens associated with the disease. Firstly, asthma is a prominent health issue among children (12, 13). Global studies have shown that only a small proportion of children with asthma have good disease control, with the situation being worse in China (14), especially among school-age children (15). Poor asthma control can lead to decreased lung function during the crucial period of lung development and function transition in childhood (16). Additionally, due to the ongoing growth and development of children, recurrent asthma exacerbations can result in growth retardation and reduced quality of life (17), and become important reasons for school absenteeism among school-age children (18, 19). Moreover, compared to their peers, school-age children with asthma are more likely to experience behavioral problems, learning difficulties, anxiety, and other issues (20). Secondly, there is currently no effective cure for asthma, and long-term, standardized, scientific, and effective disease management is necessary (21). In developed countries, the financial burden of asthma is relatively high, accounting for 1.00–2.00% of healthcare expenditures in countries with available medical expenditure data (22, 23). According to data from the US National Medical Expenditure Panel Survey from 2007 to 2013, the total annual medical expenses for school-age children with asthma amounted to 5.92 billion US dollars (22). In China, 37.80% of families with children with asthma bear annual medical expenses exceeding 10,000 yuan, and 27.60% of children with asthma have annual medical expenses exceeding 5,000 yuan (24). Thus, asthma poses a significant economic burden on both families and society (19, 25). Studies have also found that parents of children with asthma experience varying degrees of anxiety and depression during the long-term caregiving process (26). 81.52% of caregivers reported high parenting stress and psychological distress (27), 67.57% of caregivers experienced varying degrees of depression, and 29.00% of caregivers were diagnosed with post-traumatic stress disorder (27, 28).

In summary, asthma has various and significant impacts on school-age children and their parents. It is important to pay attention to the caregiver burden of parents of school-age children with asthma. However, current research mainly focuses on the caregiver burden of primary caregivers of children aged 0–14 with asthma (29–32), and there is a lack of studies specifically examining the caregiver burden of parents of school-age children (6–14 years old) with asthma. Therefore, this study aims to investigate the caregiver burden of parents of school-age children with asthma through a cross-sectional survey and analyze the influencing factors. The findings will provide practical evidence for formulating targeted strategies to alleviate the caregiver burden of parents of school-age children with asthma.

2 Materials and methods

2.1 Survey subjects

Convenience sampling method was used to select parents of school-age children with asthma who visited the pediatric outpatient departments of three tertiary hospitals in Sichuan Province, China from January 2021 to July 2021 as the research subjects. Inclusion criteria: ① Parents of children diagnosed with asthma according to the diagnostic criteria of the Chinese Medical Association's Pediatric Branch Respiratory Group's "Diagnosis and Treatment Guidelines for Children's Bronchial Asthma" (33); ② Parents of children aged 6–14 years (34); ③ The parents of the children have the ability to think and express themselves in language and a certain level of reading and comprehension skills; ④ Willing to participate in this study and sign an informed consent form. Exclusion criteria: ① Children with organic diseases, mental illnesses, or other severe chronic diseases other than asthma; ② Parents with a history of mental illness or severe chronic diseases who are unable to complete the questionnaire independently; ③ Children and their parents who have experienced significant trauma in the past 3 months. This part of the study includes 9 items on general information of parents, 6 items on general information of children, and 24 items on caregiver burden inventory, for a total of 39 analyzed variables. Sample size calculation adopted Li Zheng's rough estimation method (35), with a sample size of 5–10 times the number of variables, considering a 20.00% increase in sample size for invalid questionnaires. The calculated sample size for this study was 234–468 people, with 380 included samples and 366 valid questionnaires, resulting in a questionnaire validity rate of 96.30%. This study was approved by the Ethical Committee of Deyang People's Hospital (No. 2021-04-21-K01), the Ethical Committee of Affiliated Hospital of Southwest Medical University (No. KY2021223), and the Ethical Committee of Affiliated Hospital of North Sichuan Medical College (No. 2022ER021-1).

2.2 Survey tools

2.2.1 General information questionnaire

Including general information questionnaires for children and parents. The demographic characteristics of children include age, gender, education method, course of disease, whether pulmonary function tests were performed, number of outpatient visits due to worsening of asthma in the past 3 months, number of school absenteeism days, etc. The demographic characteristics of parents include age, gender, education level, occupation, marital status, number of children, monthly income, smoking status, child's medical expenses, family history of asthma, etc.

2.2.2 Caregiver Burden Inventory (CBI)

The Caregiver Burden Inventory (CBI), developed by Novak and Guest (36) in 1989, is widely used in domestic and international caregiver research to assess the subjective burden of caregivers during the caregiving process. The CBI questionnaire used in this

study was translated and revised by Chinese scholar Yue Peng (37) in 2006. The Chinese version of the questionnaire consists of 5 dimensions: time-dependent burden (items 1–5), developmental burden (items 6–10), physical burden (items 11–14), social burden (items 15–18), and emotional burden (items 19–24), with a total of 24 items. The questionnaire uses a 5-point scoring standard, with scores ranging from 0 to 4. A score of 0 indicates "never" and a score of 4 indicates "always." The total score ranges from 0 to 96, with scores of 0 to 32 indicating mild burden, 33 to 64 indicating moderate burden, and scores above 65 indicating severe burden. The internal consistency reliability of the questionnaire is 0.92, and the Cronbach's alpha coefficients of each dimension range from 0.68 to 0.93. In this study, the internal consistency reliability of the questionnaire was 0.85, and the Cronbach's alpha coefficients of each dimension were 0.87.

2.2.3 Data collection and quality control methods

Convenience sampling method was used in this study to strictly select survey subjects according to the inclusion and exclusion criteria. In order to achieve homogenization of the study, the research team members received unified training. Before completing the questionnaire, the researchers explain it to the research subjects and obtain their written informed consent. If the research subjects had any questions during the process of filling out the questionnaire, the researchers provided on-site explanations. After completing the questionnaire, it was collected on the spot to reduce data loss. The entire data collection process lasted for 7 months.

2.3 Statistical analysis

A database was established using Excel 2007 software, and the data was organized. SPSS 25.0 statistical software was used for data analysis. If quantitative data followed a normal distribution, it was described using mean ± standard deviation (SD); otherwise, median (M) and interquartile range (P25, P75) were used. Qualitative data were described using frequencies and percentages. Single-factor analysis of variance (Mann–Whitney U test) or multiple independent samples rank sum test (Kruskal-Wallis H test) were used to analyze the association between parents' and individual factors and caregiver burden scores, depending on the number of influencing factors. Multiple logistic regression analysis was used to analyze the factors associated with high caregiver burden. The 70th and 80th percentiles of the total burden score and each dimension score were used as the cut-off points for high burden, and the variables with $p < 0.10$ in the univariate analysis were included in the model, with the model fit using the Forward: LR method. A significance level of $p < 0.05$ was considered statistically significant.

3 Results

A total of 380 questionnaires were distributed and collected, with 366 valid questionnaires and 14 invalid questionnaires (6 questionnaires had too many missing answers, exceeding 4 items; 5 questionnaires had a regular pattern of selected options; 3 questionnaires were filled out by the same caregiver). The questionnaire validity rate was 96.30%.

3.1 General information

3.1.1 General information of parents of children with asthma

The average age of parents was (34.4 ± 5.1) years. There were 79 males (21.60%) and 287 females (78.40%). In terms of education level, 53 had junior high school education or below (14.40%), 113 had high school or technical secondary school education (30.90%), 118 had college education (32.20%), and 82 had master's degree or above (22.50%). In terms of the number of children, 233 had 1 child (63.70%) and 133 had 2 or more children (36.30%) (see Table 1 for details).

3.1.2 Demographic characteristics of children

The average age of children was (7.7 ± 1.7) years. There were 203 boys (55.50%) and 163 girls (39.30%). Course of illness≤1 year 144 (39.30%), 1 ~ 2 year 141 (38.50%), ≥3 year 81 (22.10%) (see Table 2 for details).

3.2 The caregiver burden score of parents of school-age children with asthma

3.2.1 The caregiver burden score

The total caregiver burden score was 27 (17, 39) points, 148 parents with school-age children with asthma is ≥32 points, indicating that 40.43% of parents with school-age children with asthma had a moderate to high level of caregiver burden. Among them, the score for time-dependent burden dimension was 9 (6, 13) points, the score for development-restricted burden dimension was 7 (4, 11) points, the score for physical burden dimension was 5 (2, 8) points, and the score for social burden dimension was 3 (1, 6) points (see Table 3 for details).

3.2.2 Univariate analysis of the caregiver burden

There were significant differences in caregiver burden scores based on parents' gender, highest education level, number of children, occupation, family history of asthma, monthly family income, annual medical expenses for the child, child's gender, whether the child had undergone lung function tests, number of emergency visits due to asthma exacerbation in the past 3 months, and whether the child had missed school due to asthma exacerbation in the past 3 months ($p < 0.1$) (see Table 4 for details). The original analysis results of detailed data can be found in supplements.

3.2.3 Multivariate analysis of the caregiver burden

Based on the total burden score and the scores of each dimension at the 70th percentile (P70) and 80th percentile (P80), a burden score greater than P70 or P80 is defined as an excessive caregiver burden. Logistic regression models were fitted with factors with a p-value less than 0.10 in the single-factor analysis as independent variables, using whether the caregiver burden is excessive as the dependent variable. The corresponding models are referred to as model1 and model2. The P70 and P80 for the total score and scores of each dimension are shown in Table 5. Variable assignments can be found in Table 6.

Multiple factor logistic regression analysis shows: Parents' gender (female), occupation (worker), family history of asthma (no family history of asthma), monthly family income (low monthly family

TABLE 1 Demographic characteristics of children's parents ($n = 366$).

Item	N	Constituent ratio (%)
Parents' gender		
Male	79	21.60
Female	287	78.40
Parents' age (year)		
<30	48	13.10
30~	259	70.80
≥40	59	16.10
Parents' highest education level		
Junior high school education or below	53	14.40
High school or technical secondary school education	113	30.90
College education	118	32.20
Master's degree or above	82	22.50
Number of children		
1 child	233	63.70
2 or more children	133	36.30
Occupation		
Worker	52	14.20
Farmer	63	17.20
Administrative worker	44	12.00
Service industry	129	35.20
Private business owner	78	21.40
Someone smoking at home		
Yes	199	54.40
No	167	45.60
Family history of asthma		
Yes	55	15.00
No	311	85.00
Monthly family income (CNY)		
3,000 ~ 4,999	128	35.00
5,000 ~ 9,999	142	38.80
≥10,000	96	26.20
Annual medical expenses of the child (CNY)		
<3,000	122	33.30
3,000 ~ 4,999	154	42.10
≥5,000	90	24.60

TABLE 2 Demographic characteristics of children ($n = 366$).

Item	N	Constituent ratio (%)
Child's gender		
Boy	203	55.50
Girl	163	44.50
Child's age (year)		
6~	121	33.10
7~	94	25.70
8~	73	19.90
≥9	78	21.30
Child's illness course (year)		
≤1	144	39.30
1 ~ 2	141	38.50
≥3	81	22.10
Whether the child had undergone lung function tests		
Yes	340	92.90
No	26	7.10
Number of emergency visits due to asthma exacerbation in the past 3 months (time)		
0	112	30.60
1	95	26.00
2	69	18.90
≥3	90	24.50
Whether the child had missed school due to asthma exacerbation in the past 3 months		
No	198	54.10
Yes	168	45.90

income), annual medical expenses for the child (high annual medical expenses for child), number of emergency visits due to asthma exacerbation in the past 3 months (frequent emergency visits due to asthma exacerbation in the past 3 months), and whether the child had missed school due to asthma exacerbation in the past 3 months (missed school due to asthma exacerbation in the past 3 months) were independent risk factors for caregiver burden in parents of school-age children with asthma ($p < 0.05$) (see Table 7). The original analysis results of detailed data can be found in supplements.

4 Discussion

The total burden of care score was 27 (17, 39), and among 366 parents, 148 scored ≥32, indicating that over one-third of parents of school-age children with asthma experience a moderate to high level of caregiver burden in our study. The dimensions of caregiver burden were ranked as follows: time-dependence burden, developmental restriction burden, physical burden, social burden, and emotional burden. These rankings were consistent with previous studies (38), parents of school-age children with asthma face long-term uncertainty related to their child's worsening condition. Additionally, they devote significant time and effort to children with asthma, leading to physical and mental strain. The anxiety related to time constraints and the compression of parents' working hours by caregiver responsibilities exacerbate the sense of time-dependence burden.

Gender was found to be a factor influencing the time-dependence burden dimension, with mothers being at a higher risk of experiencing excessive burden compared to fathers. Several studies have shown that female caregivers bear a greater burden during long-term care for diseases (39, 40). This can be attributed to the fact that mothers are often the primary caregivers for children within the family (41, 42), investing more time and energy in their care. Furthermore, women are more prone to experiencing negative emotions such as stress and anxiety due to physiological and adaptability differences, resulting in increased physical and emotional burden (38, 42). Support systems

TABLE 3 The results of caregiver burden ($n = 366$).

Dimension	Scoring range	Min	P_{25}	P_{50}	P_{75}	Max	Sorting
Time-dependence	0 ~ 20	0	6	9	13	20	1
Developmental	0 ~ 20	0	4	7	11	20	2
Physical	0 ~ 16	0	2	5	8	16	3
Social	0 ~ 16	0	1	3	6	16	4
Emotional	0 ~ 24	0	0	1	5	24	5
Caregiver burden	0 ~ 96	2	17	27	39	96	

TABLE 4 Univariate analysis variable screening results.

Variable	Time-dependence	Developmental	Physical	Social	Emotional	Caregiver burden
Parents' gender	√	√	√	—	—	√
Parents' age	—	—	—	—	—	—
Parents' highest education level	—	—	√	—	—	—
Number of children	√	—	—	—	√	—
Parents' occupation	√	—	√	√	—	√
Someone smoking at home	—	—	—	—	—	—
Family history of asthma	—	√	—	—	√	√
Family monthly income	—	—	√	—	—	—
Annual medical expenses of the child	—	√	√	—	—	√
Child's gender	√	—	—	—	—	—
Child's age	—	—	—	—	—	—
Child's illness course	—	—	—	—	—	—
Whether the child had undergone lung function tests	—	—	—	—	√	—
Number of emergency visits due to asthma exacerbation in the past 3 months	√	√	√	√	√	√
Whether the child had missed school due to asthma exacerbation in the past 3 months	√	√	√	√	√	√

"√" represents the factors that have an impact on CBI dimensions and overall burden, with $p < 0.10$; "—" represents $p > 0.10$.

refers to the external resources that individuals can utilize, mainly referring to the material support or psychological help obtained from family, colleagues, and friends in their social life (43). Researches have shown solid support system can enhance caregivers' ability to cope with challenges (44). It is recommended that family members, especially spouses, understand and support each other, taking turns in providing care and companionship for the child (38). Two studies have shown that the caregivers' mental status affects the caregivers' burden level, and psychological intervention can reduce caregivers' sense of stress, anxiety, and loneliness, as well as reduce caregivers' burden (45, 46). So, healthcare professionals should pay attention to the psychological well-being of mothers and provide necessary psychological interventions (47) such as cognitive-behavioral therapy, family therapy, motivational interviewing, and problem-solving therapy to alleviate the physical and mental stress experienced by mothers as primary caregivers.

Family monthly income and annual medical expenses of the child were found to be factors influencing the physical burden dimension. Wang Jing et al. (48) also identified economic issues as the main influencing factors of caregiver burden among family caregivers. This can be explained by the fact that asthma, as a chronic disease, requires financial support for its treatment and care. In order to ensure the continuity and effectiveness of their child's treatment, parents often choose to give up their jobs to take care of their child full-time (49), or they may increase their work hours or take on multiple jobs to earn more money (50). However, they still need to balance the responsibilities of supporting older adult family members and caring for their sick child, often neglecting their own health (38). This leads to excessive physical burden. It is suggested that diversified strategies be implemented at the national level, such as providing more job opportunities or flexible working hours for caregivers (44), establishing specialized medical insurance programs for children with asthma, promoting affordable medications (51), and implementing home nebulization therapy (50), in order to alleviate the economic pressure on caregivers.

The number of emergency visits due to asthma exacerbation in the past 3 months and the child's absenteeism due to asthma were found to be factors influencing multiple dimensions of caregiver burden. Several studies have indicated that recent stressful events have a negative impact on individuals' quality of life (52), which is consistent

TABLE 5 Total caregiver burden score and P70 and P80 of each dimension score.

Percentile	Caregiver burden	Time-dependence	Developmental	Physical	Social	Emotional
P_{70}	36	12	10	8	5	3
P_{80}	43	14	12	9	7	6

TABLE 6 Multivariate logistic regression analysis variable assignment table.

Independent variable	Assignment			
Dependent variable				
Is the burden too high	1 = yes	0 = normal		
Independent variable				
Parents' gender	1 = male	2 = female		
Parents' highest education level	1 = junior high school education or below	2 = high school or technical secondary school education	3 = college education	4 = master's degree or above
Number of children	1 = 1 child	2 = 2 or more children		
Parents' occupation	1 = worker	2 = farmer	3 = administrative worker	
	4 = service industry	5 = private business owner		
Family history of asthma	0 = no	1 = yes		
Monthly family income	1 = 3,000 ~ 4,999 (CNY)	2 = 5,000 ~ 9,999 (CNY)	3 = 10,000 or above (CNY)	
Annual medical expenses for the child	1 = 3,000 or below (CNY)	2 = 3,000 ~ 4,999 (CNY)	3 = 5,000 or above (CNY)	
Child's gender	1 = boy	2 = girl		
Whether the child had undergone lung function tests	0 = no	1 = yes		
Number of emergency visits due to asthma exacerbation in the past 3 months	0 = time	1 = 1time	2 = 2 times	3 = 3 times or above
Whether the child had missed school due to asthma exacerbation in the past 3 months	0 = no	1 = yes		

All multi-class variables are included in dummy variable form.

TABLE 7 Multivariate logistic regression analysis results summary.

Independent variable	Time-dependence	Developmental	Physical	Social	Emotional	Caregiver burden
Parents' gender	√	—	—	—	—	—
Parents' occupation	—	—	√	√	—	√
Family history of asthma	—	—	—	—	√	—
Monthly family income	—	—	√	—	—	—
Annual medical expenses for the child	—	—	√	—	—	√
Number of emergency visits due to asthma exacerbation in the past 3 months	—	√	√	—	—	√
Whether the child had missed school due to asthma exacerbation in the past 3 months	√	—	—	√	√	—

"√" represents the factors that influence the burden of CBI in each dimension and overall, i.e., $p < 0.05$; "—" represents $p > 0.05$.

with the findings of Jiang Di (53). In this study, the stressful events experienced by caregivers were "the number of emergency visits due to asthma exacerbation in the past three months" and "the number of school absences due to asthma in the past three months." This can be attributed to the greater caregiver difficulties faced by parents of children with asthma compared to parents of healthy children. Asthma exacerbations lead to frequent leaves from work to visit hospitals, deal with delays in the child's education, and handle school absences, which inevitably sacrifices the caregiver's rest time and social activities (54). Hospitals should pay more attention to children with frequent hospitalizations and poor disease control, as well as their family caregivers, providing targeted advice and recommendations to reduce the frequency of disease relapses (30). Additionally, weekend specialist asthma clinics should be increased (55), and the development of internet hospitals should be accelerated to expand online services (56). It is also important to establish communication platforms for caregivers, such as WeChat or QQ groups, organizing parent–child activities, and peer support meetings

(44, 57), to facilitate the sharing of caregiver experiences and promote better care for the child.

5 Conclusion

This study reveals that parents of school-age children with asthma experience a certain level of caregiver burden, with over one-third of parents experiencing moderate to high levels of burden. Being a mother, being a worker, having no family history of asthma, having low monthly family income, having high annual medical expenses for the child, having frequent emergency visits due to asthma exacerbation in the past 3 months, and having missed school due to asthma exacerbation in the past 3 months are independent risk factors for caregiver burden in parents of school-age children with asthma, healthcare providers should develop feasible coping strategies, such as paying attention to caregivers' psychological condition to reduce the burden of caring for parents of school-age children with asthma. The entire society should also make efforts in improving social support and strengthening healthcare coverage in order to achieve the aforementioned goals.

6 Strengths and limitations

The impact of asthma on school-age children and their parents is multifaceted and significant. As the primary caregivers of school-age children with asthma, parents bear a series of burdens brought about by the disease. However, current research primarily focuses on the caregiver burden of parents of children with asthma aged 0–14. This study is the first to investigate the caregiver burden of parents of school-age children (6–14 years old) with asthma in China, and of course, this study has limitations such as a limited range of research tools. In the future, more research tools should be used and more variables should be included to further improve the research results.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary materials, further inquiries can be directed to the corresponding authors.

Ethics statement

This study was approved by the Ethical Committee of Deyang People's Hospital (No. 2021-04-21-K01), the Ethical Committee of Affiliated Hospital of Southwest Medical University (No. KY2021223), and the Ethical Committee of Affiliated Hospital of North Sichuan Medical College (No. 2022ER021-1). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

FY: Supervision, Project administration, Data curation, Writing – review & editing, Writing – original draft. JZ: Software, Methodology, Data curation, Writing – review & editing. HX: Investigation, Writing – review & editing, Data curation. XW: Writing – review & editing, Investigation, Data curation. YC: Writing – review & editing, Investigation, Data curation. HH: Writing – review & editing, Investigation, Data curation. SZ: Writing – original draft, Supervision, Project administration, Formal analysis, Writing – review & editing. HL: Project administration, Writing – review & editing, Supervision, Formal analysis, Conceptualization.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We thank all the participants in this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1368519/full#supplementary-material

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frontiers | Frontiers in Public Health

TYPE Perspective
PUBLISHED 06 June 2024
DOI 10.3389/fpubh.2024.1408246

OPEN ACCESS

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RECEIVED 27 March 2024
ACCEPTED 28 May 2024
PUBLISHED 06 June 2024

CITATION
Brockway M (2024) The role of antibiotic exposure and the effects of breastmilk and human milk feeding on the developing infant gut microbiome.
Front. Public Health 12:1408246.
doi: 10.3389/fpubh.2024.1408246

The role of antibiotic exposure and the effects of breastmilk and human milk feeding on the developing infant gut microbiome

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The World Health Organization (WHO) recommends exclusive breastfeeding for the first 6 months of life followed by complementary foods and sustained breastfeeding for at least 2 years, underscoring its pivotal role in reducing infant mortality and preventing various illnesses. This perspective delves into the intricate relationship between breastfeeding practices, early life antibiotic exposure, and infant gut microbiome development, highlighting their profound influence on child health outcomes. Antibiotics are extensively prescribed during pregnancy and childhood, disrupting the microbiome, and are related to increased risks of allergies, obesity, and neurodevelopmental disorders. Breastfeeding is a significant determinant of a healthier gut microbiome, characterized by higher levels of beneficial bacteria such as *Bifidobacterium* and lower levels of potential pathogens. Despite widespread recognition of the benefits of breastfeeding, gaps persist in healthcare practices and support mechanisms, exacerbating challenges faced by breastfeeding families. This highlights the pressing need for comprehensive research encompassing breastfeeding behaviors, human milk intake, and their impact on infant health outcomes. Additionally, promoting awareness among healthcare providers and families regarding the detrimental effects of unnecessary formula supplementation could facilitate informed decision-making and bolster exclusive breastfeeding rates. Moreover, donor human milk (DHM) is a promising alternative to formula, potentially mitigating disruptions to the infant gut microbiome after antibiotic exposure. Overall, prioritizing breastfeeding support interventions and bridging research gaps are essential steps towards improving child health outcomes on a global scale.

KEYWORDS

breastfeeding, human milk, donor human milk, microbiome, antibiotics, early life exposure

Introduction

The World Health Organization (WHO) recommends exclusive breastfeeding for the first 6 months of life followed by the introduction of complementary foods and sustained breastfeeding for 2 years and beyond (1). It is estimated that scaling up rates of optimal breastfeeding can prevent 823,000 child deaths globally per year (2). Infants who are breastfed have reduced rates of acute infection, asthma, obesity, diabetes, lower and upper respiratory illnesses, and acute otitis media (ear infections) (2). Unlike formula, the composition of human milk is dynamic, and it changes throughout a feed, throughout the

day, and across the lactation stage (3, 4). While breastmilk provides a complete source of macro- and micro-nutrients for full-term infants, it also contains many non-nutritive bioactive components, such as hormones, immunoglobulins, growth factors, cytokines, microbes, metabolites, and human milk oligosaccharides that impact infant health (5). Many of these bioactive components directly influence the developing infant microbiome. In fact, infant feeding method, particularly breastfeeding and human milk feeding, is one of the most influential aspects on the developing infant microbiome (6). As such, promoting and protecting breastfeeding and offering exclusive human milk diets, even for full-term infants, may help to mitigate potential microbiome mediated risks to child health and development.

Antibiotic use and exposure in perinatal and pediatric populations

Antibiotics are the most prescribed medication during pregnancy and childhood, accounting for approximately 80% of prescriptions (7–9). A Swedish population based-cohort study found that across 125,106 pregnancies, 25.9% of mothers received antibiotics during pregnancy and over 40% of children were prescribed antibiotics in their first 2 years of life (10). Similarly in Canada, an examination of the Quebec Pregnancy Registry found that 24.5% of mothers used antibiotics during pregnancy. Antenatally (during labour), approximately 15–40% of mothers are exposed to antibiotics for Group B *Streptococcus* prophylaxis (11).

In the infant and pediatric populations from high income setting, antibiotic prescription rates in acute care exceed 35%, many in the first 30 days of life (9), whereas antibiotic prescription rates exceed 45% in outpatient care (12). As such, close to half of all children will be exposed to antibiotics during critical windows of development. While antibiotics are an important anti-infective agent, their use over the past several decades has become wide spread, often being used as a prophylaxis to prevent infection (9). It was previously believed that antibiotic exposure posed minimal risk, however as a medical community, we have come to learn that exposure to antibiotics is a concern from a resistance perspective as well as the impact that antibiotics can have on commensal microbiota in the human body (13).

Early life antibiotic exposure and child health outcomes

Early life antibiotic exposure has been associated with poorer health outcomes for children and adults. A systematic review of 160 studies examining outcomes of over 22 million children found that children who were exposed to antibiotics early in life have significantly increased risks of developing atopy and allergy, obesity and overweight, and neurodevelopmental disorders such as autism and attention deficit hyperactivity disorder (14). Additionally, it is likely that there is a dose response with increased antibiotic exposure yielding an increased risk in developing adverse health outcomes (10). It is well supported that many of these disorders may be related to the disruptions that antibiotics create in the infant microbiome during critical windows of child development.

The first 1,000 days of life, from conception to 2 years of age, is the critical period when the bacterial composition of the infant's gut microbiome helps to shape its developing immune system (15). A dysbiotic, or imbalanced, gut microbiome during critical windows of infant development in the first 1,000 days can have long-term negative consequences on child health outcomes such as metabolic diseases, asthma and allergy, and altered neurodevelopment (16). Ideally, the infant gut microbiome is established when the mother transmits microbes to their infant during vaginal delivery, followed by exclusive breastfeeding and no antibiotic treatment (15). However, more than half of full-term infants experience deviations from this cascade, including interventions such as c-section delivery (17), formula feeding (18, 19), and antibiotic exposure (7–9) which can adversely impact their developing microbiome (20).

Exposure to antibiotics in the perinatal and neonatal period significantly alters the developing infant gut microbiome, leading to a disrupted or dysbiotic state (21). Antibiotic use during pregnancy significantly disrupts the gut and vaginal microbiomes of mothers, reducing the α diversity of the bacteria that infants are exposed to when they are born vaginally, thereby impacting the initial colonization of the infant microbiome (22). Intrapartum antibiotic exposure, usually for Group B *Streptococcus* prophylaxis, is associated with a lower relative concentration of Bifidobacteriaceae, and increased relative abundance of *Proteobacteria* in the gut microbiome of exposed infants compared with those who are not exposed (21). Exposure to antibiotics during infancy significantly alters the composition of the microbiome in the developing gut, resulting in reduced levels of essential anaerobic bacteria like *Bifidobacteria*, *Lactobacilli*, and *Bacteroides*, as well as diminished populations of butyrate-producing families such as Bifidobacteriaceae, Bacteroidaceae, and Eubacteriaceae (23). Additionally, maternal use of antibiotics while breastfeeding significantly alters the breastmilk microbiome and the antibiotics can pass through the breastmilk to impact the infant's developing microbiome as well (23, 24).

Evidence consistently suggests that antibiotic exposure during pregnancy, breastfeeding and in the neonatal period, reduces colonization and abundance of important commensal bacteria such as *Bifidobacterium* (25). *Bifidobacteria* are important early colonizers of the gut because they are primary consumers of prebiotic oligosaccharides in human milk, they crowd out pathogenic bacteria, and their presence is associated with decreased risk of atopy and other childhood diseases (26).

Can breast milk and human milk feeding help to recover the infant gut microbiome?

After birth, breastfeeding is one of the most influential factors on the developing infant gut microbiome. Breastfed infants consistently demonstrate higher levels of *Bifidobacterium* compared to infants who receive formula (27, 28). Compared to exclusively formula fed infants, exclusively breastfed infants have lower α diversity which remains stable over the first 3 months of life and increases at 6 months (28). Conversely, formula fed infants demonstrate increased α diversity early in life, which is reflective of the gut microbiome in older children (28). Further, breastfed infants have increased levels of commensal bacteria such as *lactobacilli* and *enterococci*, and reduced levels of

pathogenic bacteria such as *Clostridium perfringens*, *Klebsiella oxytoca*, and *Enterococcus faecalis* (6).

Human milk contains pro-, pre- and post-biotic substances that directly impact the developing infant microbiome. Raw human milk, fed directly from the breast contains live bacteria, or probiotics, consisting of more than 800 bacterial species, that can colonize the infant gut (29). An exclusively breastfed infant consumes 1×10^5 and 1×10^7 bacteria per day (30), making human milk the second most important source of colonization of the infant gut microbiome, following bacterial exposure via the birth canal (29). Processing of human milk, such as decanting, storage, refrigeration, freezing and pasteurization can drastically impact the viability of probiotics in human milk (31, 32). While direct breastfeeding confers the greatest levels of probiotic exposure, previously refrigerated and frozen milk also still demonstrates viable microbial activity (Figure 1). Pasteurized donor human milk (DHM) has minimal microbial activity (32), and while not sterile, is not considered a viable source of probiotics.

Human milk also contains pre-biotics in the form of human milk oligosaccharides (HMOs) which are the third most prevalent component in human milk (33). There are over 150 different HMO structures in human milk and only about 1% are absorbed into the circulation of the infant (34, 35). The remaining 99% are believed to be metabolised by gut microbes or excreted by the infant (34). The primary role of HMOs is to serve as a pre-biotic for commensal bacteria in the infant gut (35). While HMOs do not naturally occur in formula, 2′-FL (2′-Fucosylactose) and LNnT (Lacto-N-neotetraose) are approved to be added to infant formulas in the US and Canada (35). Processing of human milk does not significantly reduce the pre-biotic activity and pasteurized DHM has similar levels of HMOs compared to raw human milk and is an excellent source of pre-biotics (36) (Figure 1).

Finally, human milk contains post-biotics, which are metabolites produced from microbial fermentation. Post-biotics in human milk consist of microbial cells, cell constituents and metabolites such as short-chain fatty acids (37). Presence of post-biotics may inhibit the growth of pathogens in the infant gut, enhance intestinal barrier function and mucosal immunity, and promote gene transcription (37). A consensus statement on the definition of post-biotics was only recently developed by the International Scientific Association of Probiotics and Prebiotics (ISAPP) in 2021 (38) and as such, rigorous research in this field is just emerging (37). However, it appears that processing of human milk has minimal impact on many post-biotic components and pasteurized DHM remains a good source (39).

Specific to antibiotic exposure, Dai et al. (40) found that any breastfeeding enriched *B. longum infantis* in the infant gut. This study determined that infants who were exposed to antibiotics and who were receiving any breastmilk at 3 months of age had a significantly reduced risk of developing asthma and that this relationship was mediated by *Bifidobacterium longum* Subspecies *infantis (Bifidobacterium infantis)* which is a bacterium that is dependent on human milk (40). They summarized that most species in the infant microbiome that were affected by antibiotics are responsive to breastfeeding and can be rescued to non-antibiotic levels in breastfed infants. This is one of the first studies to examine the reparative effects of breastfeeding on the microbiome of infants who are exposed to antibiotics. However, due to power limitations, they were not able to examine the effects of exclusive or direct breastfeeding on the infant microbiome.

Forms of breastfeeding – does it matter?

It is likely that the form of breastfeeding (exclusive breastfeeding compared to combo/mixed feeding or direct

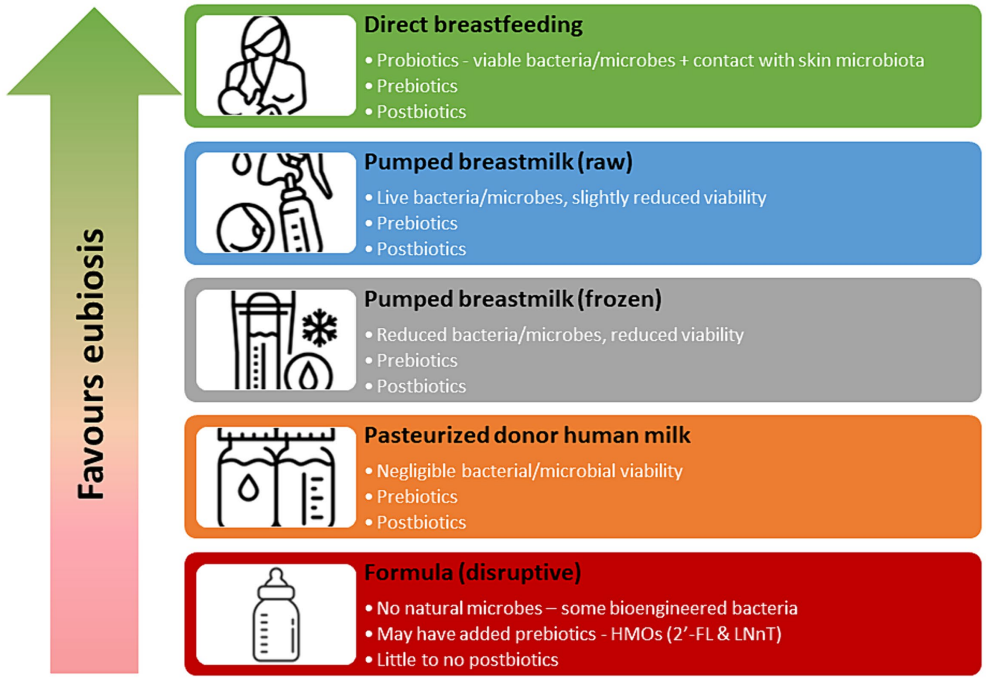

FIGURE 1
Biotic properties of human milk and formula based on mode of feeding.

breastfeeding compared to feeding expressed milk) may have varying impacts on the infant microbiome. Exclusive breastfeeding is defined as the infant only receiving breastmilk with no other fluids or complementary foods (1) and the WHO recommends exclusive breastfeeding for the first 6 months of life. Observational evidence indicates that exposure to small amounts of formula in the first days of life can significantly impact the composition of the microbiome at 3-months of age (18). Despite exclusive breastfeeding after leaving the hospital, infants who were fed formula in hospital had lower relative abundance of *Bifidobacteriaceae* in the gut microbiome at 3 months postpartum compared to infants who were exclusively breastfed throughout (18). Exclusive breastfeeding also appears to have a recovering effect on the microbiome of infants who are born via caesarean section. Liu et al. (41) found that infants born via caesarean section who were exclusively breastfed at 6 months of life had gut microbiomes that resembled infants who were born vaginally. This association was not observed for infants who received both formula and breastmilk. Unfortunately, Liu et al. (41) did not indicate if the infants who were exclusively breastfed at 6 months had ever received formula previously.

Duration of exclusive breastfeeding also appears to have an important impact on the developing infant gut microbiome. A recent systematic review and meta-analysis demonstrated a pooled protective effect of longer duration of exclusive breastfeeding and reduced risk of developing asthma for children under 7 years of age (42). Infants who are exclusive breastfed for longer periods of time are 19% less likely to develop asthma compared to children who were exclusively breastfed for shorter periods of time. Further, comparing infants who were ever breastfed to never breastfed did not yield significant differences in asthma risk, indicating that exclusive breastfeeding, or lack of formula exposure is most impactful on infant health outcomes (42). This is an important distinction as it highlights the importance of breastfeeding exclusivity, but also the duration of exclusive breastfeeding as well. While research around exclusive breastfeeding is scant, plagued by poorly defined outcomes, and highly heterogenous, it is evident that a dose response for exclusivity and duration of exclusivity exists.

Mode of breastfeeding also appears to play an important role in infant health, likely mediated via the developing infant gut microbiome. Observational evidence from the CHILD study indicates that infants who are directly fed from the breast compared to infants who are fed their mother's expressed milk have significantly reduced risk of asthma diagnoses at 3 years of age (43). Further, milk from parents who express rather than directly breastfeed demonstrates lower bacterial richness and this richness also differs between milk that is pumped compared to milk that is manually expressed (44). It is likely that the varied richness of the milk microbiota directly informs the colonization of the infant gut, as up to 30% of the infant microbiome is derived from breastmilk intake (45). Despite mode of breastfeeding, it is important to note that both direct and expressed feeding of breastmilk confers a significant risk reduction of asthma diagnoses compared to formula feeding (43).

It is evident that gut microbiome signatures are heavily influenced by infant feeding type (28, 46) and breastfeeding can improve gut microbiota composition in infants who experience adverse early life exposures (47). It also appears that exclusive breastfeeding and direct breastfeeding may provide enhanced protection for infants who experience adverse early life disruptions to their microbiome such as antibiotic exposure. However, future research in child health needs to carefully measure exclusivity of breastfeeding, considering if there has been any previous formula exposure and how the breastmilk is being provided to the infant.

Clinical and research considerations

Promotion and protection of breastfeeding and exclusive human milk feeding is a public health intervention that is affordable and effective in improving child health outcomes on a global scale (2). However, there still exist some gaps in the evidence and many areas of healthcare lack evidence-based practice strategies to support breastfeeding families. Further, as evidence of breastfeeding benefits have started to accumulate over the past 20–30 years, public and health discourse around infant feeding has increasingly become very pro-breastfeeding and mothers are under intense social pressure to exclusively breastfeed their infants (48, 49). Yet, society provides minimal support or choice of supplementation when breastfeeding is not possible or does not go as planned.

Considerations for research

Breastfeeding and human milk intake has long been under reported and not accurately measured in child health research. Further, breastfeeding evidence is predominantly limited to observational studies exploring associations between breastfeeding and infant health outcomes, which have been critiqued for their risk of confounding (48). While the WHO and most healthcare agencies recommend exclusive breastfeeding for the first 6 months of life (1), rigorous evidence to support these guidelines is still lacking. Increasingly, we are observing the profound impact and protective effect that breastfeeding and human milk feeding has on infant development and child health outcomes (2). It is likely that this relationship is mediated by the role human milk has on the developing gut microbiome in infants (46). However, conclusive evidence about this relationship is lacking. Future research needs to include careful measurement of breastfeeding behaviors and human milk intake. Collecting data on exclusivity of breastfeeding, mode of breastfeeding, amount of supplementation, and type of supplementation will provide much stronger evidence around the relationship between infant feeding and infant health. In addition, prioritizing the relationship between infant feeding and the developing infant microbiome in research will help to provide a better understanding of the mechanism and dose response of human milk feeding on the developing infant microbiome.

Provider education

There have been many calls to action to further protect and promote breastfeeding. These have been relatively successful in increasing breastfeeding initiation rates to >90% in Canada (50), and to >85% in the United States (51). However, over 60% of mothers do not meet their breastfeeding goals (52) and exclusive breastfeeding rates fall well below the recommended guidelines (50, 51). The most common reasons for formula supplementation in the early days of life are related to medical indications, such as

hypoglycemia, weight loss, or jaundice; parental request or preference; and lactation management issues, such as poor latch or perceived insufficient milk supply (53). In addition, it is still common for healthcare providers to defer to formula without sufficient medical indication or providing adequate support to breastfeeding families (53, 54). Providing mandatory education for healthcare providers who work with families in the perinatal setting is an evidence-based strategy that helps to improve breastfeeding rates (2, 54, 55). Additionally, raising awareness among healthcare providers and families around the unethical marketing of breastmilk substitutes from formula companies may help to enhance critical thinking around unnecessary formula supplementation and increase capacity for informed decision making with infant feeding (56).

Supplementation options

Exclusive breastfeeding rates still lag far behind recommended guidelines (50, 51). It is estimated in Canada and the United States, 40–60% of full-term infants receive at least one bottle of formula supplementation in their first week of life (18, 19, 51). Not only does this disrupt the infant microbiome (18), but it also disrupts the establishment of breastfeeding and leads to lower breastfeeding exclusivity and duration rates (57, 58). Donor human milk (DHM) is likely a superior alternative to formula when supplementation is required because it allows infants who are supplemented to continue to be exclusively fed human milk. Through its various biotic properties, DHM minimizes perturbations to the gut microbiome in infants who experience adverse early-life exposures (59, 60). As such, there are likely profound differences in the impact of formula supplementation compared to DHM supplementation on the developing infant gut microbiome (36, 61). Additionally, evidence indicates that mothers who supplement with DHM compared to formula may be more likely to continue exclusively breastfeeding their infants at 6 months (62). However, there is minimal research on the impact of DHM as a supplementation option for the full-term population and on the full-term infant gut microbiome (63). While DHM is the accepted standard of practice for preterm infants when supplementation is indicated in clinical settings, little is known about DHM use in the full-term infant population. Research in this area is laden with opportunity to establish causal relationships between DHM supplementation (exclusive human milk feeding) compared to formula supplementation on the infant gut microbiome. Randomization of infants to receive DHM instead of formula is still an ethical practice because DHM supplementation is not standard practice in the full-term population. DHM is a viable and feasible alternative to formula supplementation that should be considered for full-term infants who experience early life perturbations to their microbiome.

Conclusion

The interplay between breastfeeding, early life antibiotic exposure, and infant gut microbiome development underscores the critical importance of promoting and protecting breastfeeding and human milk feeding for optimal child health outcomes. While lifesaving, unnecessary antibiotic exposure and overuse is a significant concern and may pose long-term risks to child health. Breastfeeding is associated with a myriad of benefits, including a healthier gut microbiome and reduced risks of infections and diseases and may help overcome some of the perturbations to the microbiome from antibiotic exposure. However, current breastfeeding rates lag behind WHO recommendations. Addressing these challenges requires a multi-faceted approach, including comprehensive research to elucidate the mechanisms underlying breastfeeding's protective effects and the impact of interventions like DHM supplementation. Additionally, healthcare providers must prioritize breastfeeding support and education to empower families in making informed decisions regarding infant feeding. By bridging research gaps, enhancing breastfeeding support mechanisms, and raising awareness about the advantages of exclusive breastfeeding and DHM supplementation, we can strive towards improving child health outcomes and fostering a healthier future generation. Ultimately, investing in breastfeeding promotion and support initiatives is a cost-effective public health intervention that deserves commitment and dedication from policymakers, healthcare providers, and society as a whole.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

MB: Conceptualization, Investigation, Methodology, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author declares that financial support was received for the research, authorship, and/or publication of this article. MB holds a Canadian Institutes of Health Research – Canada Research Chair in Early Life Exposures and Child Health. MB is also funded by the Social Sciences and Humanities Research Council.

Conflict of interest

MB serves on the board of directors for the NorthernStar Mothers Milk Bank, for which she does not receive remuneration.

Publisher's note

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frontiers | Frontiers in Allergy

TYPE Original Research
PUBLISHED 27 August 2024
DOI 10.3389/falgy.2024.1456077

OPEN ACCESS

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RECEIVED 27 June 2024
ACCEPTED 08 August 2024
PUBLISHED 27 August 2024

CITATION
Mamun A, Zou C, Lishman H, Stenlund S, Xie M, Chuang E and Patrick DM (2024) Association between antibiotic usage during infancy and asthma incidence among children: a population-level ecological study in British Columbia, Canada.
Front. Allergy 5:1456077.
doi: 10.3389/falgy.2024.1456077

Association between antibiotic usage during infancy and asthma incidence among children: a population-level ecological study in British Columbia, Canada

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Background: This study follows published associations in BC to 2014 (updated in 2019) to model the predicted incidence of asthma in BC children attributable to antibiotic use within the context of reduced antibiotic use and increased breastfeeding in BC infants from 2000 to 2019.
Methods: A population-based ecological study was conducted in BC from 2000 to 2019, using outpatient antibiotic prescription data from BC PharmaNet and asthma diagnoses from the Chronic Disease Registry. Breastfeeding estimates were calculated using the Canadian Community Health Survey (CCHS). Population attributable risk (PAR) was calculated using a blended relative risk (RR) of asthma in antibiotic-exposed children who were and were not breastfed. PAR was used to calculate predicted vs. actual asthma incidence in 2019. Negative binomial regression was used to estimate the association between the average antibiotic prescription rate in infants under 1 and asthma incidence in 1–4 year olds, stratified by periods between 2000–2014 and 2015–2019.
Results: In BC, antibiotic prescribing decreased by 77% in infants under 1 and asthma incidence decreased by 41% in children 1–4 years from 2000 to 2019. BC breastfeeding rates increased from 46% in the 2005 CCHS to 71% in the 2017/18 CCHS. After calculating the PAR using a blended RR, the predicted asthma incidence in 2019 was 18.8/1,000 population. This was comparable to the observed asthma incidence in children 1–4 years of 16.6/1,000 population in 2019. During 2000–2014, adjusted incidence risk ratio (aIRR) for children under Quintile 5 of average antibiotic prescribing was 1.75 (95% CI: 1.63–1.88, $P < 0.0001$) times higher than that for Quintile 1. However, between 2015 and 2019, this association weakened (as expected because of increasing prevalence of breastfeeding), with the expected asthma incidence for Quintile 5 only 11% (aIRR 1.11, 95% CI: 0.78–1.57) higher than for Quintile 1.
Conclusion: We identified that over the past 20 years, antibiotic exposure in infants under 1 and asthma incidence in children 1–4 years has decreased significantly. Decreasing antibiotic exposure and increasing breastfeeding (which further mitigates risk associated with antibiotics) are of sufficient scale to explain much of this population trend. Changes in environmental, social and other exposures remain relevant to this complicated etiological pathway.

KEYWORDS

asthma, antibiotics, breastfeeding, incidence, Canada

Background/Introduction

A growing body of evidence shows an association between receiving antibiotics during infancy and developing asthma and other atopic diseases in childhood (1). One potential mechanism is disruption of the developing gut microbiota, and impairment of its role in training the developing immune system away from atopy (2). A reduction in gut microbial diversity and the absence of key bacterial taxa has been associated with an increased incidence of atopic disease with evidence continuing to accrue from longitudinal cohort studies (3). Given that antibiotics can significantly affect a child's developing microbiome (4), they have been investigated as potential contributors to childhood asthma (1). Alongside prospective birth cohort studies, population-level data have shown that a significant decline in childhood antibiotic use is associated with falling asthma incidence in some jurisdictions such as British Columbia (5). This may be relevant to the plateauing of asthma incidence trends in the UK and the US after a decade of rising trends (6–8) and to significant declines in childhood asthma diagnosis in England (9) and Germany (10) in recent years.

Although antibiotics are still among the most commonly used therapeutics for children, concerns about their adverse effects on health have shifted prescription trends in some countries (4). In the Nordic countries, for example, antibiotic prescriptions for children have decreased in the past decade driven by factors such as changed guidelines for otitis media treatment and the introduction of the pneumococcal vaccine (11). Survey data from 132 countries indicated an increase in the proportion of children receiving antibiotics from 36.8% to 43.1% between the years 2005 and 2017 (12). Of these countries, low-income countries showed the greatest relative increase but still showed the lowest rates (39.5%) in 2017. While the incidence of childhood and adult asthma have peaked in many high income jurisdictions, it is still on the rise in many middle and low-income countries (13). Furthermore, asthma incidence in children is still showing a rising trend in new global cohorts of children (14). Although hospital admissions and asthma rates have generally decreased since 2000 (15, 16), these changes are primarily due to shifts in population proportions (14, 17).

Influences on the ecology of the infant gut microbial environment are complex and antibiotics are not the only factor that may play a role in microbial diversity and subsequent atopic disease risk. Other factors associated with childhood asthma include maternal smoking, obesity, infections and antibiotic use during pregnancy, prematurity, caesarean delivery, air pollution, and respiratory infections (16), most of which influence the gut microbiome. A factor of particular interest, breastfeeding, has recently been shown to have protective effects against asthma in children exposed to antibiotics (18) and could mitigate antibiotic-mediated damage to the microbiota through enrichment of *B. longum subsp. Infantis* (19). In high income countries, the proportion of babies having exposure to breastmilk before 6 months has increased between 2000 and 2019. In low income countries, this rate has slightly decreased but exclusive breastfeeding rates have increased (20). Exposure to rich microbiological load in a farm environment has also been observed to decrease allergic outcomes and respiratory disease in children (21). Overall, the *hygiene hypothesis* states that societal changes in developed countries have led to reduced early-life microbial exposures, negatively impacting immunity and increasing the risk of atopic outcomes (2).

Our previous ecological study, conducted between 2000 and 2014 in BC, Canada, revealed an association between antibiotic use before the age of 1 and incidence of asthma diagnosis at 1–4 years old (5). The current study extends the analyses to 2019 at the Local Health Area (LHA) level in BC, aiming to explore if observed associations are the same in recent years and to model the simultaneous effects of changing rates of breastfeeding on asthma incidence.

Materials and methods

We conducted a population-based ecological study to examine the association between antibiotic use in the first year of life and asthma in children aged 1–4 years of age between 2000 and 2019. The ethics approval for this study was obtained from the University of British Columbia Clinical Research Ethics Board (H09-00650).

Population-level antibiotic prescribing and asthma incidence

We obtained anonymized antibiotic prescription data from BC PharmaNet; a database that captures information on all outpatient prescriptions dispensed by community pharmacies in the Canadian province of British Columbia (population 5.1 million), except for some drugs used for HIV and STI (22). We calculated the number of prescriptions by age group, year, sex, and local health area (LHA). We obtained population estimates for the 89 LHAs of British Columbia by age group (23). We calculated the mean percentage of children exposed to one or more courses of antibiotics during infancy, mean prescription rates, total number of prescriptions by year, and cumulative percent change in prescription rates over the study duration. We retrieved 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 (24, 25). Particulate matter metrics, indexed to postal codes, and material and social deprivation indices were provided by the Canadian Urban Environmental Health Research Consortium (sourced by the Atmospheric Composition Analysis Group at Dalhousie University, Halifax, Canada) (26, 27).

Modeling expected fall in asthma incidence due to reduction in antibiotic use and changes in prevalence of breastfeeding using population attributable risk

To model the impact of decreasing exposure to antibiotics in children <1 year and increasing prevalence of breastfeeding on

asthma incidence in 1–4 year olds, we calculated the population attributable risk (PAR). PAR is the proportion of disease in a population that would not occur if a risk were removed. When relative risk for an exposure (RR_e) and the proportion exposed (P_e) are known, it is derived as follows:

$$PAR = Pe\,(RRe - 1)/\,[1 + Pe\,(RRe - 1)]$$

First, we modeled the expected asthma incidence rate (ages 1–4 years) in the BC population in 2019, given the observed reduction in P_e (proportion of the infant population <1year of age prescribed one or more courses of antibiotics) under various assumptions for the relative risk associated with antibiotic exposure. Because breastfeeding has been shown to reduce the RR associated with antibiotic exposure, we also modeled a blended relative risk which accounted for the prevalence of breastfeeding. To calculate the change in the proportion of mothers who partially breastfed their last child in BC, we used two different cycles from the Canadian Community Health Survey (CCHS): 2005 and 2017/18 (28). Due to the unavailability of data, the breastfeeding rates for 2000 and 2019 were approximated using CCHS data from 2005 to 2018 respectively, which were the closest years for which data was available. The proportion of breastfeeding outcome from the 2017/18 cycle was determined by Chan et al. (29). The Public Use Microdata Files (PUMFs) were used from the CCHS Maternal Experiences (MEX) module to derive the breastfeeding outcome from the 2005 cycle, available from Statistics Canada through the Abacus Data Network (30). To calculate the proportion of mothers who exclusively or partially breastfed their last child for a duration of at least 6 months, the following equation was used (31): Total weighted number of women 15–55 years giving birth in past 5 years and who breastfed their child ≥6 months/Total weighted number of women 15–55 years giving birth in the past 5 years × 100.

Dai et al. reported adjusted odds ratios of asthma incidence in children who were exposed to antibiotics with breastfeeding (adj. OR of asthma incidence = 1.31) and who were exposed to antibiotics without breastfeeding (adj. OR of asthma incidence = 3.53) in the first year of life (19). Using these estimates, we calculated the antibiotic exposure for each of the breastfeeding exposures considered in this study for calculating the PAR:

$$RR(abx) = Pe(BF) \times 1.31 + (1 - Pe(BF)) \times 3.53$$

This formula is a weighted average of odds ratios for antibiotic exposure with and without breastfeeding, representing a blended relative risk (RR).

$$\begin{aligned}&\text{change in asthma incidence attributable to antibiotics}\\ &= PAR(2000) \times \text{baseline incidence} - PAR(2019)\\ &\times \text{baseline incidence}\end{aligned}$$

$$\begin{aligned}&\text{predicted asthma incidence (2019)}\\ &= \text{baseline incidence} - \frac{\text{change in asthma incidence}}{\text{attributable to antibiotics}}\end{aligned}$$

Association between antibiotic exposure and asthma incidence using negative binomial modeling

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 89 LHAs within British Columbia. Negative binomial models were used (as the equidispersion assumption was violated when exploring whether a Poisson regression model could be used). We built a multivariable negative binomial model to estimate the association between the rate of antibiotic prescribing at the LHA-level in infants <1 and asthma incidence at age 1–4 years. The primary independent variable was the antibiotic prescription rate quintile during the first year of life, calculated by categorizing prescription data into five equal-frequency groups based on distribution. The dependent variable was asthma incidence for children aged 1–4 years. Covariates were sex, material and social deprivation indices, and the mean concentration of fine particulate matter [<2.5 μm (PM2.5)] annually in each LHA. We incorporated the LHA as a random effect to account for unmeasured geographical variability. The data was merged from the British Columbia Ministry of Health Chronic Disease Dashboard, BC PharmaNet database, and population estimates data by year, LHA, and sex. Upon observing a changing pattern of antibiotic prescription rates over time, we stratified the data into older years (2000–2014, results of which are published) and recent years (2015–2019) to better capture the differences in the association between asthma incidence and antibiotic prescription quintiles in these periods. We fitted separate negative binomial models for each time period, enabling the analysis of the relationship between asthma incidence and antibiotic prescription quintiles within each timeframe. We performed statistical analysis using R software (version 3.5.2).

Results

At the population level in British Columbia, from 2000 to 2019, the annual incidence of asthma in children aged 1–4 years showed an absolute decrease of 11.6 new cases per 1,000 children, from 28.3 (27.4, 29.0) in 2000 to 16.6 (16.0, 17.2) per 1,000 children in 2019, a relative decrease of 41% (Figure 1). Between 2000 and 2014, there was an absolute decrease of 8.7 cases per 1,000 children, falling from 28.3 (95% CI 27.5–29.1) cases in 2000 to 19.5 (95% CI 18.9–20.2) cases in 2014, a relative decrease of 31%. However, after 2014, the rate of decrease in asthma incidence in children appears to be stabilising.

In 2000, for children 1–4 years of age, the corresponding 4-year mean annual antibiotic prescription for infants (aged <1 year) was 1,183.7 per 1,000 infants. This rate decreased to 276.74 in 2019, an absolute decrease of 906.96 per 1,000 infants and a relative decrease of 77% (Figure 1). The average proportion of infants exposed to one or more courses of antibiotics before the age of 1 year

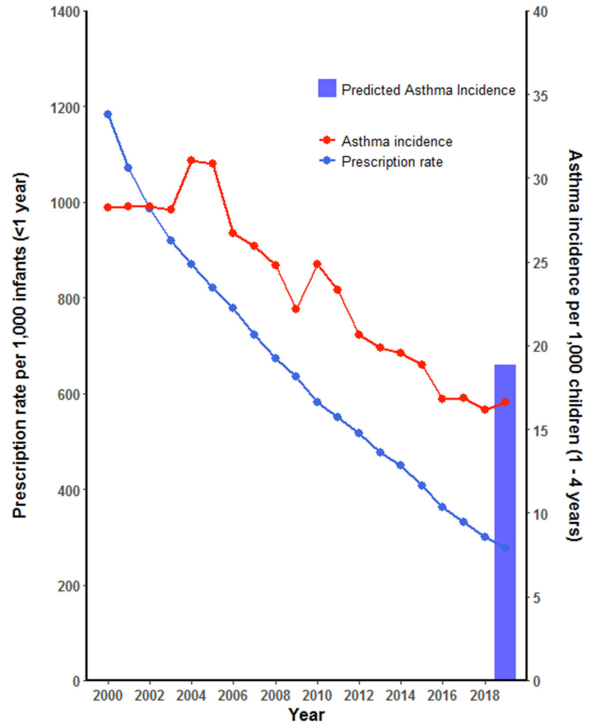

FIGURE 1
Asthma incidence in BC children aged 1–4 years and 4-years average antibiotic prescription rate during first year of life, 2000–2019. Predicted asthma incidence in BC children using PAR in 2019.

decreased by 46%, from 66% in 2000 to 20% in 2019 (Figure 2), representing a cumulative relative decrease in annual antibiotic prescription rate of 76% (Figure 3). The average proportion of infants exposed to one or more courses of antibiotics before the age of 1 year decreased from 30% in 2014 to 20% in 2019 (Figure 2). While we observed a decrease in asthma incidence during the study period, we also observed a decrease in asthma prevalence during the same period (Supplementary Figure 1). However, similar to the incidence of asthma, the prevalence of asthma has also stabilised during the most recent years of the study. Asthma incidence in children (1–4 years of age) and antibiotic use in the first year of life were strongly correlated (Spearman's $r = 0.9489$, $p < 0.001$). Amoxicillin was the most frequently prescribed antibiotic for infants across all years of the study, making up 6,916 (68·5%) of 10,096 antibiotic prescriptions in 2019 (Supplementary Table 1).

Predicting asthma incidence with various levels of exposure to antibiotics and breastfeeding

Following the population attributable risk calculation and using a range of hypothetical relative risk scores, we modelled expected asthma incidence after observing the decrease in antibiotic exposure. With the reduction in antibiotic exposure and a relative risk (RR) of asthma of 2, we would predict an annual asthma incidence decrease between 2000 and 2019 from 28.3 per 1,000 children to 22.3 per 1,000 children. With a relative risk of asthma to be 3, we would predict an annual asthma incidence decrease between 2000 and 2019 from 28.3 per 1,000 children to 21.0 per 1,000 children. However, the actual observed asthma incidence in this study decreased to 16.6 per 1,000 children in 2019 (Figure 1).

In our final model to predict asthma incidence with the antibiotic exposure reduction, we included the modelled changes in breastmilk exposure. Using the equation outlined in the methods, the proportion of BC mothers who exclusively or partially breastfed their child for a duration of at least 6 months in the 2005 CCHS cycle was $386/831 \times 100 = 46\%$. And in the 2017/18 cycle of the CCHS, Chan et al. calculated 70.7% of respondents in BC reported breastfeeding their child born in the last 5 years (either exclusively or partially) for 6 months or more (29). We calculated the relative risk of asthma incidence associated with antibiotic exposure and the corresponding breastfeeding exposure using the method as described in the methods section. For breastfeeding exposure of 46% in 2005 (as a proxy for exposure in 2000) and the antibiotic exposure observed in 2000, the blended relative risk of asthma incidence was calculated as 2.53 (Table 1). For breastfeeding exposure of 71% in 2018 (as a proxy for exposure in 2019) and the antibiotic exposure observed in 2019, the blended relative risk of asthma incidence was calculated as 1.98. Using a decreased relative risk of asthma incidence from 2.53 in 2000 to 1.98 in 2019 and an increase in breastfeeding exposure from 0.46 in 2005 to 0.71 in 2018, we predicted the annual asthma incidence to decrease from 28.3 per 1,000 children in 2000 to 18.8 per 1,000 children in 2019. The above predicted annual asthma incidence was very close to the observed annual asthma incidence (16.6 per 1,000 children in 2019) when incorporating changes in both antibiotic and breastmilk exposures (Figure 1).

Association between antibiotic exposure and asthma incidence

Over the study period both the minimum and maximum average annual prescription rate per 1,000 children per year decreased, mostly observed during the latter years (Table 2). Using the negative binomial model, we calculated adjusted incidence rate ratios (aIRRs) predicting asthma incidence and observed a significant association between the quintiles of average annual antibiotic prescription rate and asthma incidence for the period from 2000 to 2014. Expected incidence of asthma for children under Quintile 5 (antibiotic prescription range 1,072.01–3,982.04 per 1,000 infants) was estimated to be 75% (aIRR 1.75, 95% CI: 1.63–1.88, $P < 0.0001$) higher than that for Quintile 1 (antibiotic prescription range 30.97–331.67 per 1,000 infants) (Figure 4). We observed a dose-response relationship when looking at asthma risk for the children under other Quintiles of prescribing compared to Quintile 1. However, during the period of 2015–2019, this association appeared to be weakened, with the expected asthma incidence for Quintile 5

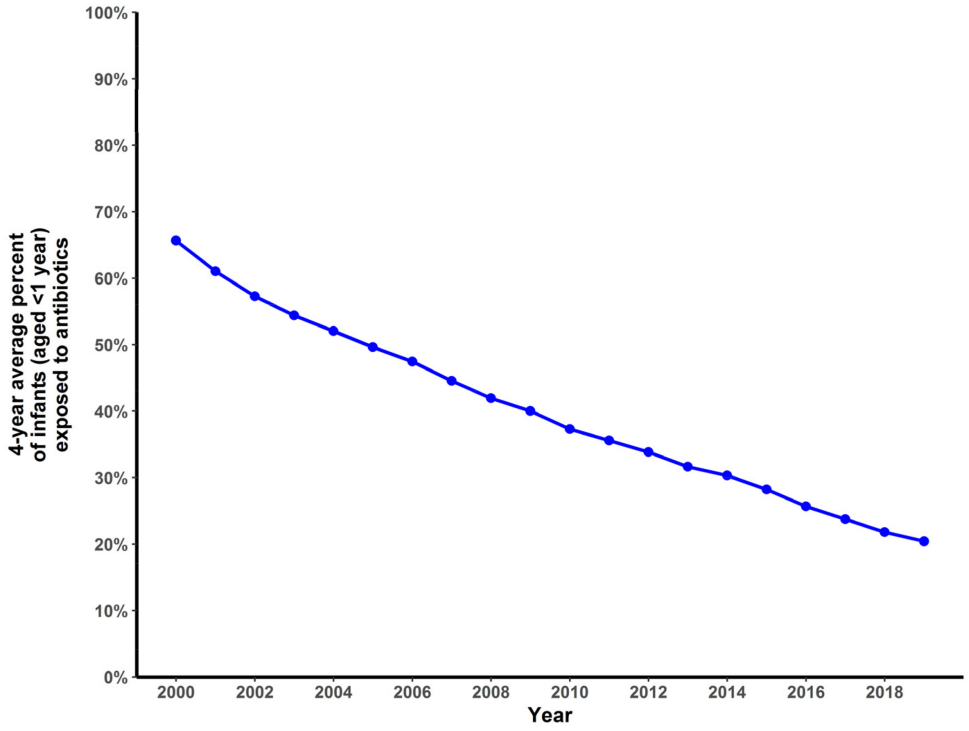

FIGURE 2
Four-year average percentage of BC infants (<1 year) who received one or more antibiotic prescriptions in their first year of life, 2000–2019.

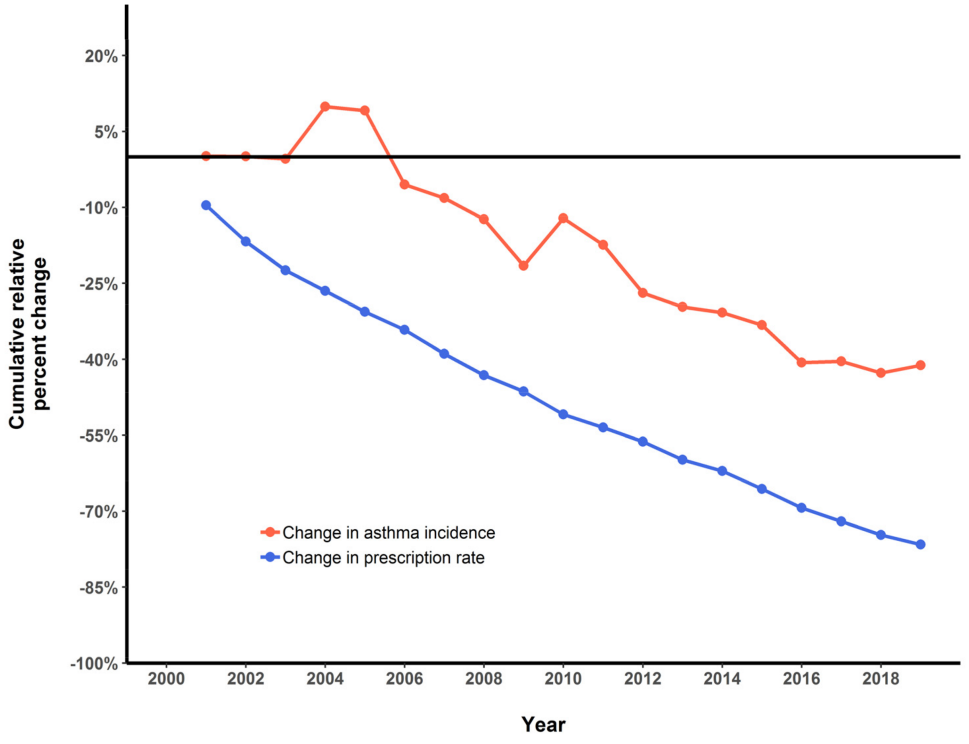

FIGURE 3
Cumulative relative percent change in 4-year average antibiotic prescription rate (<1 year of age) and asthma incidence (1–4 year of age) in BC children, 2000–2019.

TABLE 1 Counterfactual model to demonstrate the effect of adding breastfeeding to the PAR model.

Scenario	P(antibiotics)	P(breastfed)	Blended RR	Asthma incidence
2000 baseline (observed)	66%	46%	2.53	28.3/1,000 pop
2019 counterfactual model	20%	46%	2.53	22.2/1,000 pop
2019 model (observed)	20%	71%	1.98	18.8/1,000 pop

TABLE 2 Four-year average prescription rate ranges of infants (<1 year) in BC, 2000–2019.

Year	Min	Max
2000	387.9	3,817.3
2001	327.5	3,696.2
2002	280.0	3,914.9
2003	187.3	3,982.0
2004	143.2	3,931.6
2005	161.7	3,790.1
2006	148.3	3,552.2
2007	128.3	3,184.0
2008	172.1	3,061.5
2009	154.2	2,864.2
2010	185.5	2,678.0
2011	135.7	2,833.9
2012	102.4	2,565.1
2013	130.4	2,646.6
2014	140.5	2,901.7
2015	111.5	2,881.1
2016	135.7	2,822.7
2017	115.9	2,240.8
2018	86.5	1,840.0
2019	31.0	1,781.3

only 11% (aIRR 1.11, 95% CI: 0.78–1.57) higher than for Quintile 1 (Figure 5), and this difference was not statistically significant. Moreover, we didn't observe a dose-response relationship for asthma risk as observed for the duration of 2000–2014. We initially hypothesized that the observed weakening association between antibiotic prescription rates and asthma incidence in recent years (2015–2019) might be attributed to a reduction in the highest rates of antibiotic prescriptions, particularly within Quintile 5. To test this hypothesis, we performed additional analyses controlling for extreme antibiotic prescription rates. Even after excluding prescription rates above a threshold of 2,881 per 1,000 infants (which represents the maximum prescription rate in the recent years, 2015–2019) (Table 2), the association between antibiotic prescription rates and asthma incidence in the older years (2000–2014) remained stronger than in the recent years (Data not shown). We found that boys had more than 50% higher risk of asthma in both time periods of this study (Figures 4, 5).

Discussion

In our population-based study of 5.1 million people in British Columbia, we noticed a 41% decrease in asthma incidence between 2000 and 2019 in children 1–4 years of age, which correlated with a 77% decrease in the corresponding antibiotic prescription rate in the first year of life. The observed time gap between the decrease in antibiotic exposure and decrease in asthma incidence is likely reflecting the 4 years required to gather data on a full cohort of children 1–4 years of age. Patrick et al. reported a 26% decrease in asthma incidence between 2000 and 2014 in a similar study in BC, Canada (5). Our study extended the study period and explored if the previously observed decline in asthma incidence in BC is consistent during the recent years (2015–2019). We observed that asthma incidence has stabilised between 2015 and 2019 with the exposure to one or more courses of antibiotics before the age of 1 year having decreased from 30% in 2014 to 20% in 2019. We also found a similar trend when looking at the asthma prevalence in BC for the same duration. However, there was a strong correlation between asthma incidence in children aged 1–4 years and antibiotic exposure during the first year of life.

In addition to a decrease in antibiotic exposure, other factors such as reductions in air pollution and an increase in breastfeeding exposure are likely to have contributed to the observed associations (5, 32). In this study, we modelled the expected incidence of asthma with a range of relative risk estimates. This method assumed the decrease in antibiotic exposure in infancy and increase in breastfeeding exposure were major independent predictors of asthma incidence at the population level. We couldn't explain most of the observed decrease in asthma incidence in this study, when the model included antibiotic exposure only. However, when modelled using both the observed decrease in antibiotic exposure and available data on breastfeeding exposure, we predicted the asthma incidence of 18.8 per 1,000 children in 2019, which is very close to the observed rate (16.6 per 1,000 children in 2019). Therefore, higher rates of breastfeeding in BC in recent years is likely another contributing factor to why the true asthma incidence rate was lower than the predicted asthma incidence when only antibiotic prescribing was considered. We used partial breastfeeding exposure without accounting for breastfeeding duration. Previously, Patrick et al. reported no significant association between breastfeeding duration and asthma risk. However, authors hypothesized that breastfeeding could act as a effect modulator and may regulate the gut during antibiotic disruption, restoring bacterial diversity and largely mitigating the risk of asthma (5). Moreover, Dai et al. reported clear protection from the risk of developing asthma when children are breastfed while exposed to antibiotics (19).

In our study, we calculated an aIRR predicting asthma incidence, and observed a significant association between the quintiles of average annual antibiotic prescription rate in the first year of life and asthma incidence in children 1–4 years of age. However, when the aIRRs was calculated for 2000–2014 and 2015–2019 separately, we observed that the aIRR is weaker

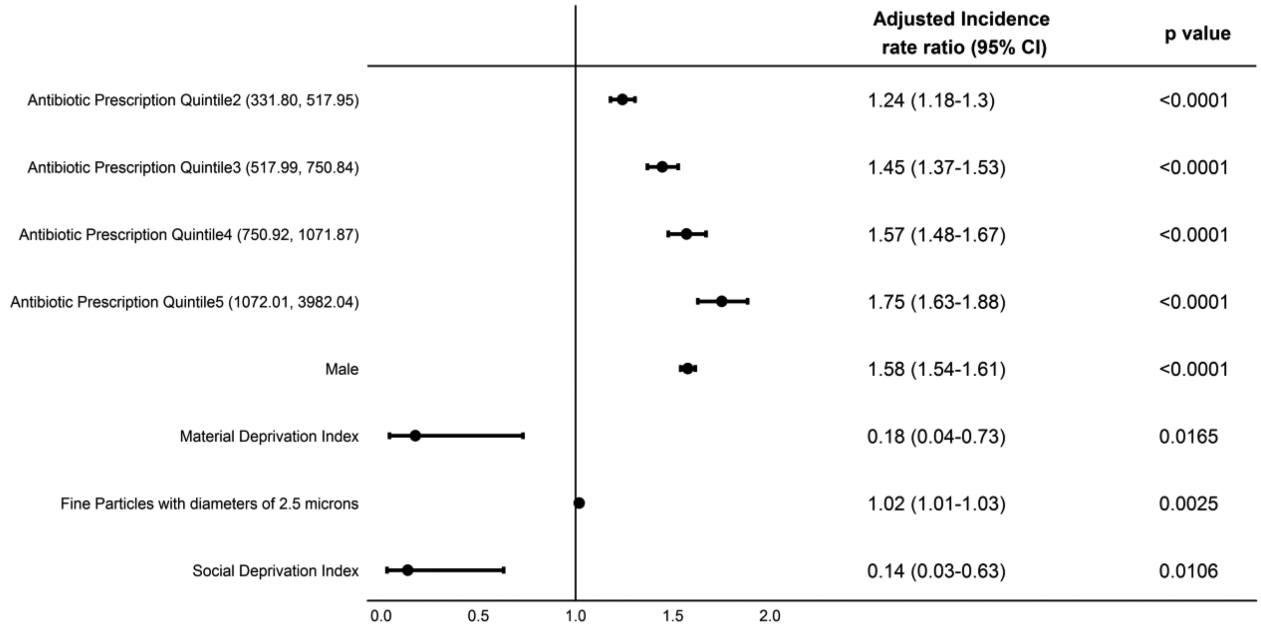

FIGURE 4
Adjusted incidence rate ratios (aIRRs) predicting asthma incidence in BC children aged 1–4 years, 2000–2014.

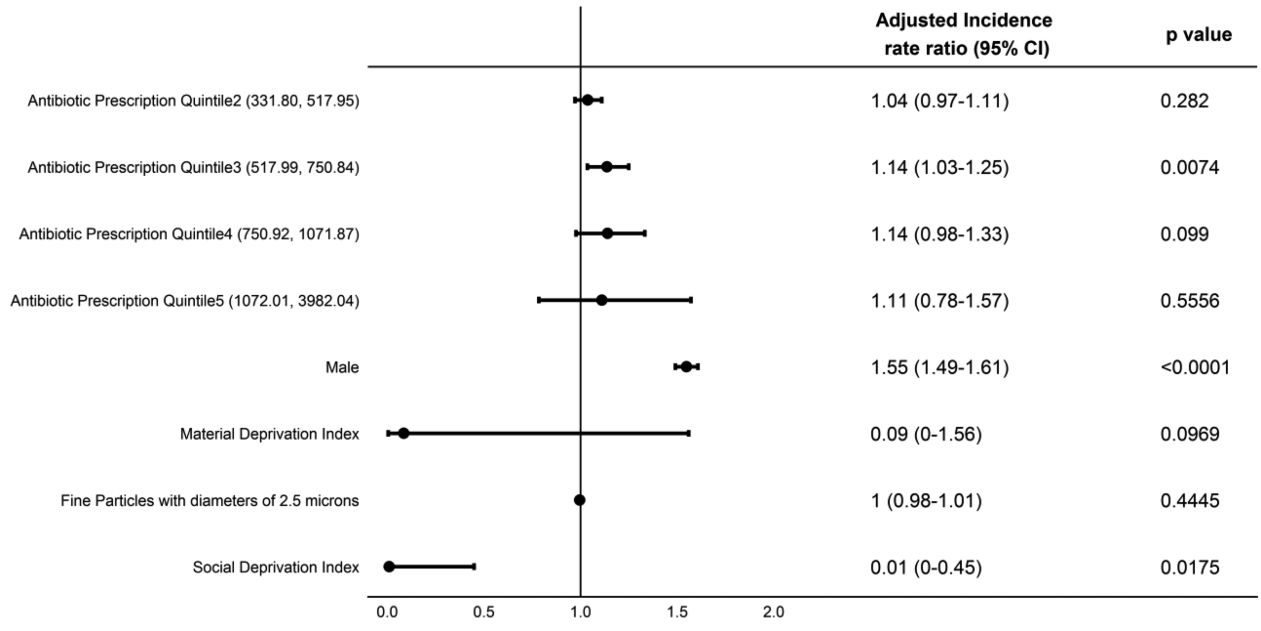

FIGURE 5
Adjusted incidence rate ratios (aIRRs) predicting asthma incidence in BC children aged 1–4 years, 2015–2019.

compared to that during 2000–2014. A lower relative risk associated with antibiotics is what we would predict in a population with more exposure to breast feeding. This finding along with the modelled expected asthma incidence may suggest that decreases in antibiotic exposure may not be the only major factor in decreasing asthma risk and that there are other factors playing a role in reducing asthma risk, such as breastfeeding. Dai et al. reported that compared with children with no antibiotic exposure in the first year of life, antibiotic-exposed children who were not breastfed had 3-fold higher odds of developing asthma, whereas there was no such association in antibiotic-exposed children who were breastfed (19).

Limitations

This ecological study has several limitations. Due to unavailability, we were not able to use the breastfeeding exposure data for the same year as for the antibiotic exposure and the breastfeeding data that we used for predicting the asthma incidence was based on the Canadian Community Health Survey which has its own limitations. The actual breastfeeding exposure might be different during the years for which antibiotic exposures were included in the model of expected asthma risk. Moreover, we couldn't analyze the effect of various forms of breastfeeding such as exclusive or partial breastfeeding or pumped breast milk. We couldn't analyze the effect of individual antibiotic classes on asthma risk and our data didn't allow us to account for indications given for antibiotic use. For the asthma diagnoses, we relied on the BC chronic disease registry database which rely on clinical diagnostic codes which may lead to some misclassification. This study was designed to address some of the gaps identified in our previous ecological study and to observe if the previous finding is still consistent with the more recent years of data. However, there are many details or gaps which are not possible to address through the ecological analysis. Patient-level factors that were outlined in the introduction cannot be adjusted for using this design, of particular importance being the indication for antibiotic prescribing, and therefore inferences cannot be drawn at the patient-level. In recognition of this limitation, and to build upon this work, we have designed a population-based cohort study comprising all infants born in BC between 2001 and 2011, following up to age 7, with the aim of evaluating the effect of reducing antibiotic use on asthma, allergic rhinitis and atopic dermatitis (33). This will provide an opportunity to account for variations at the individual-level and offer insights into subpopulations receiving antibiotics for different indications to thoroughly investigate and adjust for potential confounding by indication. It will also allow for an investigation into whether different types of antibiotics confer differential risk of atopic outcomes.

Conclusion

We identified that over the past 20 years antibiotic exposure in infancy has decreased significantly in BC, as has asthma incidence in children 1–4 years of age, with asthma incidence stabilising in more recent years. Asthma risk might not be explained by only antibiotic exposure. Air pollution and other social and economic factors as well as breastfeeding during early childhood are important factors to account for when analysing asthma risk in children.

Data availability statement

The data analyzed in this study is subject to the following licenses restrictions: access to data provided by the Data Stewards is subject to approval but can be requested for research projects through the Data Stewards or their designated service providers. Requests to access these datasets should be directed to https://healthdataplatformbc.ca. The following data sets were used in this study: Chronic Disease Registry and Pharmanet. All inferences, opinions, and conclusions drawn in this publication are those of the author(s), and do not reflect the opinions or policies of the Data Steward(s). The following BC Ministry of Health datasets were used in this study: Chronic Disease Registry and Pharmanet. All inferences, opinions, and conclusions drawn in this publication are those of the author(s), and do not reflect the opinions or policies of the Data Steward(s).

Ethics statement

The study was approved by the UBC Clinical Research Ethics Board (H09-00650). The study was conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

AM: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. CZ: Data curation, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. HL: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. SS: Methodology, Writing – original draft, Writing – review & editing. MX: Data curation, Software, Visualization, Writing – original draft, Writing – review & editing, Formal Analysis. EC: Data curation, Software, Visualization, Writing – original draft, Writing – review & editing, Formal Analysis. DP: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The population level analyses were made possible by funding (funding letters 1224499 and 1253861) from the British Columbia Ministry of Health - Clinical Services & Evaluation - Pharmaceutical, Laboratory and Blood Services Division (Victoria, BC, Canada).

Acknowledgments

We would like to acknowledge Dr. Fawziah Marra for her input on the DINPIN list to define antibiotic utilization. Access to data provided by the Data Stewards is subject to approval but can be requested for research projects through the Data Stewards or their designated service providers. The following data sets were used in this study: Chronic Disease Registry, Pharmanet, BC

Stats Population Estimates and CANUE. All inferences, opinions, and conclusions drawn in this publication are those of the author(s), and do not reflect the opinions or policies of the Data Steward(s). PM2.5 metrics, indexed to DMTI Spatial Inc. postal codes, were provided by CANUE (Canadian Urban Environmental Health Research Consortium). The population level analyses were made possible by funding from the British Columbia Ministry of Health, Pharmaceutical Services Division (Victoria, BC, Canada).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/falgy.2024.1456077/full#supplementary-material

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frontiers | Frontiers in Allergy

TYPE Systematic Review
PUBLISHED 07 October 2024
DOI 10.3389/falgy.2024.1458688

OPEN ACCESS

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RECEIVED 03 July 2024
ACCEPTED 06 September 2024
PUBLISHED 07 October 2024

CITATION
Wurm J, Curtis N and Zimmermann P (2024) The effect of antibiotics on the intestinal microbiota in children - a systematic review. Front. Allergy 5:1458688.
doi: 10.3389/falgy.2024.1458688

The effect of antibiotics on the intestinal microbiota in children - a systematic review

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Background: Children are the age group with the highest exposure to antibiotics (ABX). ABX treatment changes the composition of the intestinal microbiota. The first few years of life are crucial for the establishment of a healthy microbiota and consequently, disturbance of the microbiota during this critical period may have far-reaching consequences. In this review, we summarise studies that have investigated the effect of ABX on the composition of the intestinal microbiota in children.
Methods: According to the PRISMA guidelines, a systematic search was done using MEDLINE and Embase to identify original studies that have investigated the effect of systemic ABX on the composition of the intestinal microbiota in children.
Results: We identified 89 studies investigating a total of 9,712 children (including 4,574 controls) and 14,845 samples. All ABX investigated resulted in a reduction in alpha diversity, either when comparing samples before and after ABX or children with ABX and controls. Following treatment with penicillins, the decrease in alpha diversity persisted for up to 6–12 months and with macrolides, up to the latest follow-up at 12–24 months. After ABX in the neonatal period, a decrease in alpha diversity was still found at 36 months. Treatment with penicillins, penicillins plus gentamicin, cephalosporins, carbapenems, macrolides, and aminoglycosides, but not trimethoprim/sulfamethoxazole, was associated with decreased abundances of beneficial bacteria including Actinobacteria, *Bifidobacteriales*, *Bifidobacteriaceae,* and/or *Bifidobacterium*, and *Lactobacillus*. The direction of change in the abundance of *Enterobacteriaceae* varied with ABX classes, but an increase in *Enterobacteriaceae* other than *Escherichia coli* was frequently observed.
Conclusion: ABX have profound effects on the intestinal microbiota of children, with notable differences between ABX classes. Macrolides have the most substantial impact while trimethoprim/sulfamethoxazole has the least pronounced effect.

KEYWORDS

microbiome, stool, intestine, sequencing, 16S rRNA, penicillin, cephalosporin, macrolide

Introduction

Children are the age group with the highest exposure to antibiotics (ABX). ABX are the second most prescribed drugs for children, surpassed only by analgesics (1–5). More than two-thirds of children receive ABX before reaching the age of two years (6) with exposure to an average of almost three ABX in the first year of life (7). Approximately one third of

hospitalised children (8) and nearly half of acutely ill children in outpatient settings receive ABX (9), often with inappropriate indications or drugs (10). While the widespread use of ABX has significantly reduced childhood morbidity and mortality during the last century (11), exposure to ABX is also associated with adverse long-term health effects. These include an increased risk for atopic dermatitis, allergies, wheezing and asthma, obesity, arthritis idiopathic disorder, psoriasis, and neurodevelopmental disorders (12). These adverse effects likely result from changes in the microbiota, particularly the intestinal microbiota, which undergoes significant development during the first two to three years of life (13–15). Children are highly susceptible to ABX-induced dysbiosis (an imbalance in the microbiota), whereas adults typically have a more stable and resilient microbiota that recovers more easily from such disturbances (16). The first years of life are critical for the development and stabilisation of the intestinal microbiota, coinciding with key milestones in the development of the immune system, metabolism and neurodevelopment (17–19). Consequently, any disturbances of the microbiota during this critical period may have long-lasting and far-reaching consequences.

In this review, we systematically summarise studies that have investigated the effect of ABX on the composition of the intestinal microbiota in children of all age groups.

Methods

In May 2024, MEDLINE (1946 to present) and Embase (1972 to present) were searched. Embase was searched using the OVID interface. The detailed search terms can be found in the supplementary data. No geographical limitations were used. References of retrieved articles were searched for additional publications.

Original studies which investigated effect of ABX on the composition of the bacterial intestinal microbiota in children less than 18 years of age were included, as well as studies involving mixed-age populations that provided separate data specifically for this age group. Exclusion criteria were studies which (i) included children with underlying diseases (e.g., oncological diseases, cystic fibrosis) and (ii) studies not published in English, German, French, Spanish, Portuguese or Italian.

The following variables were extracted from included studies: year of publication, country, study design, number and characteristics of included children, number of samples, ABX treatment (drug, dose, frequency, route of administration, duration), previous ABX, microbiota analysis method, timing of stool analysis and key findings (including changes in diversity, abundance of microbes, antibiotic resistance genes, ARGs).

Studies were identified, selected, appraised, and synthesised following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for systematic reviews (20, 21). The level of evidence of each study was classified according to the 2011 Oxford Centre for Evidence-Based Medicine (OCEBM) Levels of Evidence (22). Risk of bias was assessed using the 2017 Joanna Briggs Institution (JBI) standardised critical appraisal checklist for case-control and cohort studies (23).

Results

The search identified 6,146 and 11,470 studies in MEDLINE and Embase, respectively. From the 17,616 studies, 2,183 duplicates were removed. 89 studies met the inclusion criteria (24–112). No additional relevant studies were found through citation searching. The selection of included studies is summarized in Figure 1.

The 89 studies investigated a total of 9,712 children (of these 4,574 were controls; mean 111 children per study, range 9 to 1,023) and 14,845 samples (mean 322 samples per study, range 20 to 1,247). Of the included studies, 47 were done in Europe (Denmark, Netherlands, Finland, Spain, France, Italy, Germany, Ireland, Estonia, United Kingdom, Sweden, Norway, Austria) (24, 26, 28, 29, 31, 32, 34, 35, 37–39, 43, 45, 48–52, 60, 61, 63, 64, 66, 67, 70, 71, 74, 75, 81–83, 88, 92, 93, 95, 98, 101, 102, 104–112), 21 on the American continent (USA, Chile, Canada) (25, 30, 41, 42, 44, 54–56, 58, 59, 69, 73, 76, 78, 84–86, 94, 96, 97, 99), 13 in Asia (China, India, Japan, Korea, Taiwan, Lebanon) (27, 40, 53, 57, 65, 68, 72, 77, 80, 87, 90, 91, 100), seven in Africa (Niger, Burkina Faso, Zimbabwe, South Africa) (36, 46, 47, 62, 79, 89, 103) and one in Australia (33). The age of participants ranged from preterm birth to 15 years. Of the included studies, 75 were observational studies, including 60 cohort studies (24, 25, 27, 28, 30–35, 37–42, 44, 45, 48–58, 61, 64–68, 70, 71, 73, 74, 76–78, 84, 87–92, 94, 97–102, 109–112), 12 cross-sectional studies (26, 29, 43, 72, 75, 82, 85, 93, 95, 99, 108, 113), three pre-post-intervention studies (35, 60, 96); and 13 (cluster) randomised controlled trials (36, 46, 47, 59, 62, 63, 69, 79–81, 83, 86, 103). In total, 42 studies used 16S rRNA gene sequencing (25–28, 40–46, 48, 51, 52, 55, 57, 58, 62, 64, 66, 68, 69, 71–73, 76, 77, 79, 80, 83–86, 91, 92, 98–103, 113), ten shotgun metagenomic sequencing (29, 30, 36, 47, 54, 75, 83, 89, 94, 98), seven polymerase chain reaction (PCR) or PCR-temperature gradient gel electrophoresis (PCR-TGGE) (39, 49, 50, 65, 67, 78, 82), one each fluorescence *in situ* hybridization (FISH) (104) and 16S-23S IS profiling (48), and 30 cultures (24, 31–35, 37–39, 49, 53, 56, 59–61, 63, 74, 81, 87, 88, 90, 93, 96, 105, 107–112).

Supplementary Table S1 provides a summary of the main findings of the 89 studies included in this review.

All included studies had an overall risk of bias score (JBI standardised critical appraisal checklist, yes%) over 60% (acceptable quality) and 49% (44/89) of studies had an overall score ≥80% (good quality) (Supplementary Table S3). The most frequent risk of bias was attrition bias [present in 24% (21/89) studies].

Penicillins

The effect of penicillins on the intestinal microbiota was investigated in 13 studies including 1,311 children [one study did not specify the number of children investigated (70); for detailed information see Supplementary Table S3] (35, 55, 70, 71, 75, 78, 79, 87, 89, 90, 100, 107, 112).

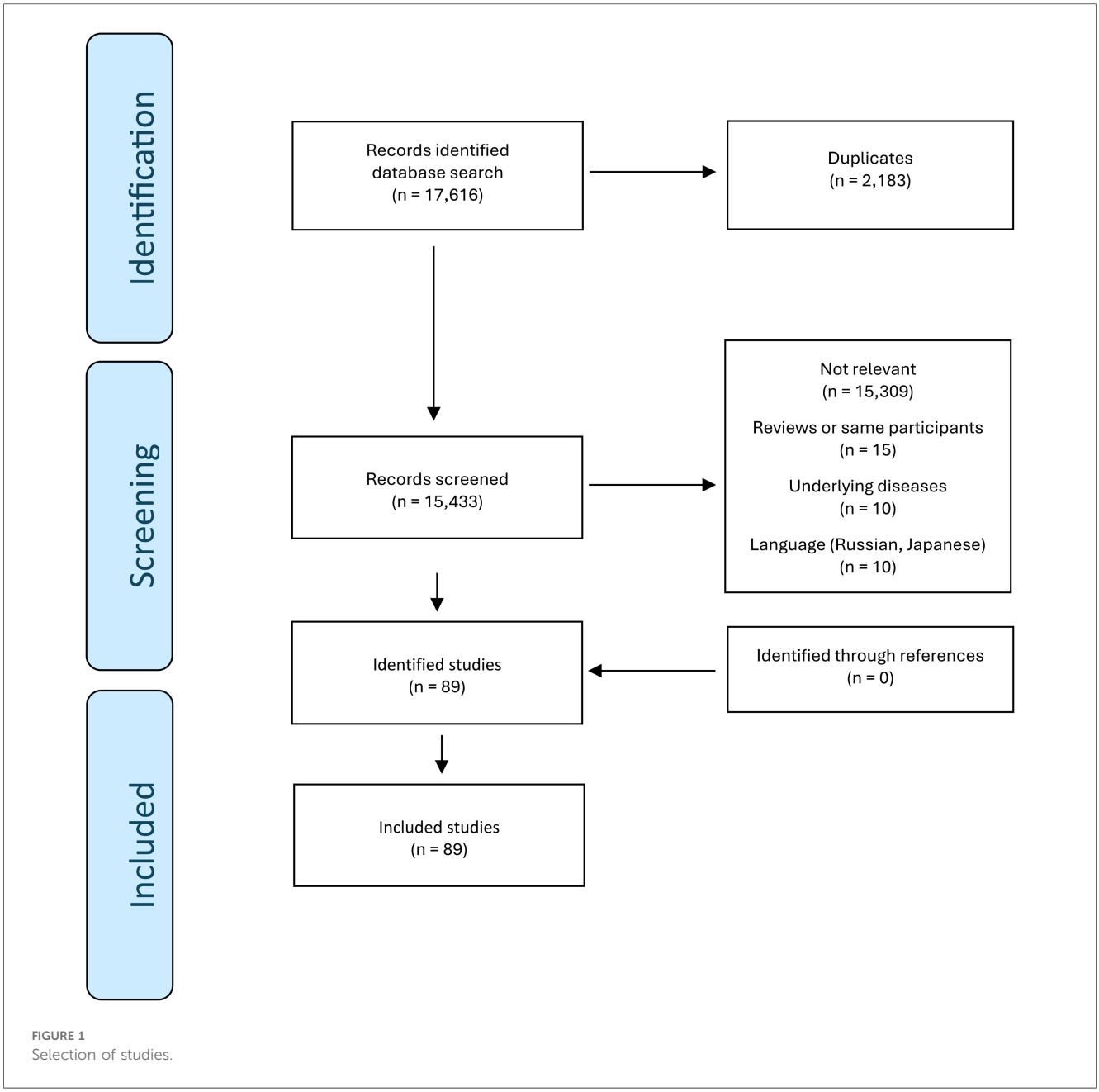

FIGURE 1
Selection of studies.

Diversity

Two studies found a lower alpha diversity (a measure of the variety and abundance of bacterial taxa within a sample) of the intestinal microbiota during the treatment with amoxicillin compared to controls with recovery either immediately after stopping ABX (89) or six months after (71), respectively. However, compared to controls, the first study found a higher alpha diversity at the latest follow-up time point 24 months after ABX (89). In other studies, compared to controls, a lower richness was found to persist for less than two weeks after treatment with ampicillin and penicillin (75), and for up to 6–12 months (latest follow-up being after 12–24 months) in a study (which did not report separate results for treatment with amoxicillin with or without clavulanate and penicillin V) (70) (Figure 2). One study found a lower alpha diversity at day seven of life compared to day three in preterm neonates after treatment with penicillin plus moxalactam or piperacillin-tazobactam, but no difference in diversity after ABX with the two treatment regimens (100). Another study found no difference in alpha diversity, at the latest follow-up point of the study, five days after treatment with amoxicillin (79). Three studies investigated beta diversity (a measure of the differences in bacterial taxa between different samples) by comparing the ABX group and controls and found a higher beta diversity after treatment with penicillin, amoxicillin/ampicillin, penicillin plus moxalactam and piperacillin-tazobactam, respectively (75, 89, 100).

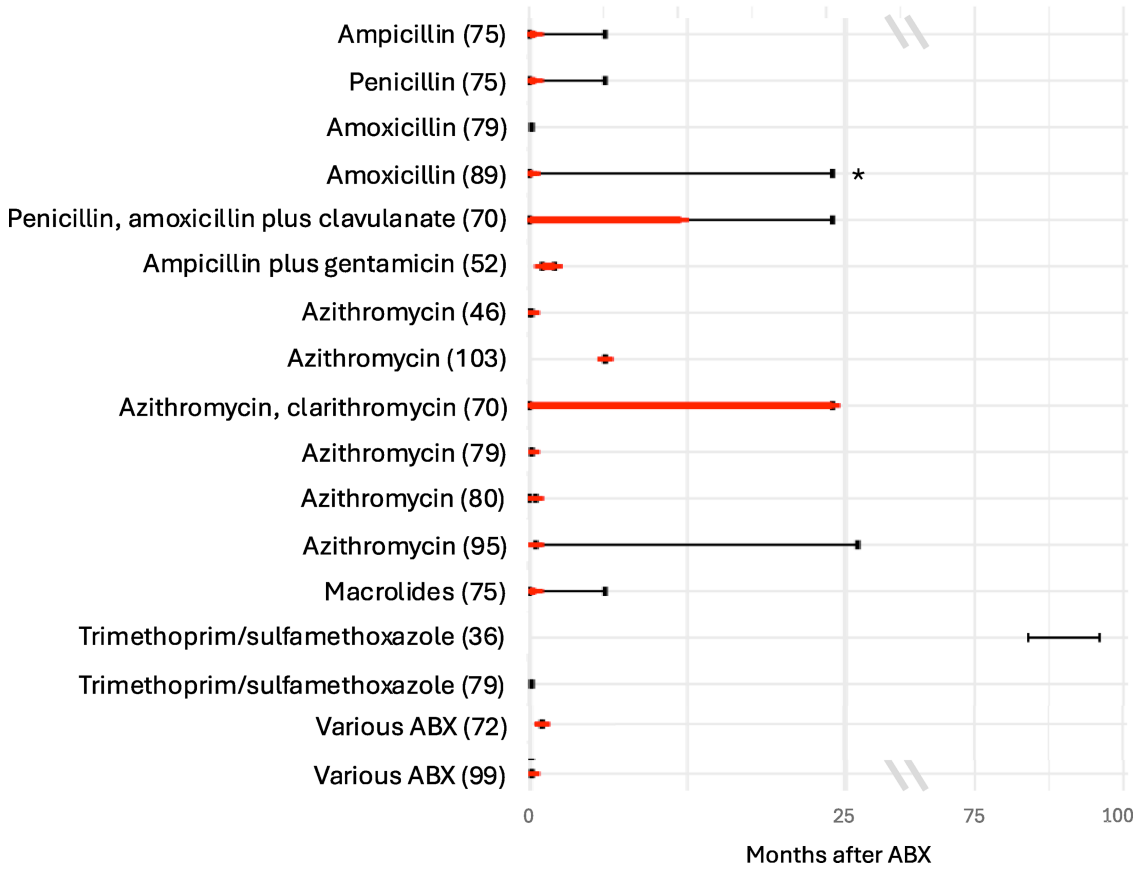

FIGURE 2
Duration of decreased alpha diversity after ABX reported in different studies. Red bars depict duration of decreased alpha diversity in ABX group compared to controls (red bars); black bars depict the duration of follow-up after ABX. Studies specifying Shannon index, inverse Simpson index or richness for the ABX and control group were included. Studies which did not specify the time points when the alpha diversity was investigated were excluded.

Composition

When comparing the composition of the intestinal microbiota during or after oral administration of amoxicillin, compared to controls, increased abundances of Firmicutes (71), Enterobacteriales (71), *Ruminococcaceae* (71), *Lachnospiraceae* (71), *Megasphaera* (71), *Coprococcus* (71), *Escherichia* (89), *Dialister* (71), *Weissela confusa* (89), *Prevotella* sp. 885 (89), *Prevotella stercorea* (89), *Holdemanella biformis* (89), *Lactobacillus animalis* (89), *Fusicatenibacter saccharivorans* (89), *Catenibacterium mitsuokai* (89), *Slackia isoflavoniconvertens* (89), *Weissella cibaria* (89), *Streptococcus macedonicus* (89), *Gemmiger formicilis* (89), and *Actinomyces odontolyticus* (89), and decreased abundances of *Coriobacteriaceae* (71), *Bacteroidaceae* (71), *Streptococcaceae* (71), *Lactobacillus* (89), *Bifidobacterium* (71), *Enterococcus* (71), *Streptococcus* (87, 89), *Klebsiella* (89), *Holdemanella* (89), *Dorea* (89), *Bifidobacterium bifidum* (89), and *Bifidobacterium longum* (89) were observed.

When comparing the composition before and during treatment with penicillin V, ampicillin and methicillin, respectively, decreased bacterial counts of *Lactobacillus* (87), *Streptococcus* (87), and *Bifidobacterium* (87) were found. One study found increased abundances of Bacteroidetes, *Rikenellaceae,* and *Dialister* and decreased abundances of Actinobacteria, *Gemellales*, *Gemellaceae, Lactobacillus,* and *Collinsella* in children who were given penicillin or amoxicillin with or without clavulanate, without reporting results for these ABX separately and without reporting the route of administration (70). Fluctuations in abundance over time were found for *Veillonellaceae* (71), *Clostridiaceae* (both being lower during treatment with amoxicillin and higher than in controls after treatment) (71) and *Parabacteroides* (being higher within less than 6 months after treatment with amoxicillin with or without clavulanic acid or penicillin (without separate analysis) compared with controls and then being lower 6 to 12 months after treatment compared with controls (70) (Figure 3). One study found no difference in bacterial abundance after treatment with penicillin (75).

ARGs

The effect of penicillins on the abundance of ARGs was studied in three studies. Two studies found an increased abundance of

FIGURE 3
Differences in bacterial abundance between children treated with penicillins and controls. Included studies investigated amoxicillin (71, 89) and amoxicillin with or without clavulanate and penicillin V (70). Studies which did not compare ABX group to controls and studies not providing *p*-values were excluded.

ARGs after treatment with penicillin and ampicillin/amoxicillin, respectively, which returned to normal three to four weeks after treatment (75, 89). The other study found increased abundances of multiple ARGs, e.g., for β-lactamases (unknown), ABC efflux pumps, *araC,* and *emrD* (55). A higher abundance of plasmids after treatment with penicillin or ampicillin (without separate analysis) was reported in another study (75).

Penicillins plus aminoglycosides

13 studies investigated combinations of different penicillins plus gentamicin in 3,141 children (for detailed information see Supplementary Table S3) (26, 37, 40, 52, 58, 63, 69, 81, 83, 85, 93, 94, 110).

Diversity

Compared with controls, three studies found a lower alpha diversity in children treated with penicillin, amoxicillin, or ampicillin plus gentamicin (52, 58, 83). The duration of treatment with ampicillin plus gentamicin positively correlated with the decrease in alpha diversity (58). One study found a lower alpha diversity in children treated for more than seven days with ampicillin plus gentamicin compared with these treated for a shorter duration (94). Another study found a lower richness in children treated with ampicillin plus gentamicin compared with controls two months after treatment (52) (Figure 2). In contrast, one study found no difference in alpha diversity at two weeks of life after treatment with ampicillin plus gentamicin (69), and another one, one year after treatment with penicillin plus tobramycin (26). Yet another study compared the alpha diversity one week after treatment with ampicillin plus tobramycin, ampicillin plus tobramycin plus metronidazole, and ampicillin plus cefotaxime and found no difference between these groups (85). Another study compared alpha and beta diversity after treatment with ampicillin plus gentamicin and ampicillin plus cefotaxime and found no differences at the latest follow up at 30 days of life (40).

Composition

Compared to controls, after treatment with ampicillin plus gentamicin increased abundances/colonisation rates of Proteobacteria (52), *Bacilli* (94), *Clostridiales* (94), *Bacteroidales* (94), *Enterobacteriaceae* (52), *Peptostreptococcaceae* (52), *Clostridium* (52), *Klebsiella* (37, 83, 94), *Enterobacter* (58, 93), *Klebsiella/Enterobacter* (93), *Veillonella* (80), *Streptococcus* (80), and *Enterococcus faecalis* (37), and decreased abundances of *Bifidobacteriacea* (52), *Bifidobacterium* (52, 83), *Escherichia* (83), *Staphylococcus* (58, 83), and *Lactobacillus* (52); after treatment with penicillin plus gentamicin increased abundances of *Acinetobacter* (83), and *Klebsiella* (83), and decreased abundances of *Bifidobacterium* (83), *Escherichia* (83), *Staphylococcus* (83), and *Escherichia coli* (83); after treatment with penicillin plus netilmicin decreased abundance of *Clostridum difficile* (63); and after treatment with penicillin plus tobramycin decreased abundances of Bacteroidetes were found (26). Conflicting results were reported for the abundance of Actinobacteria (52, 69), *Enterococcus* (52, 58, 83) and *Bacteroides* (83, 94), and *Escherichia coli* (37, 93, 94) (Figure 4).

ARGs

One study reported that changes in the ARG profile persisted for up to four months after treatment with amoxicillin/clavulanate plus gentamicin with 10 of 31 ARGs being more abundant, while after penicillin plus gentamicin five of 10 ARGs were found to be more abundant (83). One study found resistance to ampicillin in *E. coli, Klebsiella,* and *Enterobacter* after ampicillin plus gentamicin treatment (93).

Penicillins plus cephalosporins

Five studies investigated combinations of penicillins plus cephalosporins, including 1,123 children (for detailed information see Supplementary Table S3) (34, 40, 83, 85, 101).

Diversity

One study found a lower alpha diversity in children treated with amoxicillin plus cefotaxime compared with controls (83). The same study also found a high beta diversity with a dissimilar composition between children treated with amoxicillin plus cefotaxime and controls, which was still present four months after stopping ABX (83). One study compared alpha diversity one week after treatment with ampicillin plus tobramycin, ampicillin plus tobramycin plus metronidazole, and ampicillin plus cefotaxime and found no difference between these groups (85). Another study compared alpha and beta diversity after treatment with ampicillin plus gentamicin and ampicillin plus cefotaxime and found no differences at the latest follow-up at 30 days of life (40).

Composition

Increased abundances of *Enterococcus* (101), *Clostridium* (101), and *Acinetobacter* (83), and decreased abundances of *Bifidobacterium* (101), *Akkermansia* (83), and *Escherichia coli* (83) were found in children after treatment amoxicillin plus cefotaxime or ceftazidime compared to controls. One study compared abundances after treatment with ampicillin plus tobramycin, ampicillin plus tobramycin plus metronidazole, and ampicillin plus cefotaxime and found no difference between groups (85). One

FIGURE 4
Differences in bacterial abundance or colonization rate between children treated with penicillins plus aminoglycosides and controls. Studies included investigated ampicillin plus gentamicin (52, 58, 69, 81, 93, 94), penicillin plus gentamicin (81, 83), and penicillin plus tobramycin (26). Studies which did not compare ABX group to controls and studies not providing *p*-values were excluded.

study did not provide *p*-values for their analysis of colonisation rates (34).

ARGs

One study reported changes in the ARG profile with 10 of 31 ARGs being more abundant after treatment with amoxicillin plus cefotaxime. Compared to penicillin plus gentamicin (five of 10 ARGs being more abundant), amoxicillin plus cefotaxime was found to have a higher impact on ARG abundance (83).

Cephalosporins

The effect of cephalosporins on the intestinal microbiota in children was investigated in nine studies including 916 children (for detailed information see Supplementary Table S3) (55, 57, 60, 63, 87, 88, 90, 91, 93).

Diversity

One study found a lower alpha diversity in infants treated with cefalexin compared with controls at two months of life (91). Another study found a lower richness immediately after treatment with cefotaxime and cefazoline compared to before ABX (55). One study found no difference in alpha diversity in neonates at ten days of life between the ABX group and controls after treatment with cefotaxime (57). Three studies did not analyse alpha diversity or richness (60, 87, 93).

Composition

The administration of cefalexin was associated with increased abundances of *Enterobacteriaceae* (91), *Enterococcus* (91), and decreased abundances of *Bifidobacterium* (91) compared to controls. The administration of cefuroxime was associated with a decreased abundance of *Escherichia coli* (93). When comparing before and after treatment with cefaclor decreased bacterial counts of *Enterobacteriaceae* (87), *and Bifidobacterium* (87), were found, while when comparing before and after treatment with ceftazidime decreased bacterial counts of *Enterobacteriaceae* (87), *Lactobacillus* (87), and *Bifidobacterium* (87), were found. After treatment with cefotaxime, compared to controls, increased abundances of *Enterobacteriaceae* (57) and *Parabacteroides* (57), and decreased abundances of *Bifidobacterium* (57), *Clostridium difficile* (63) and *Escherichia coli* (55) were found. After treatment with ceftriaxone, abundances of *Enterobacteriaceae* and *Lactobacillus* increased compared to before ABX (88). When comparing before and after treatment with cefpiramide decreased counts of *Enterobacteriaceae* (87), *Bacteroidaceae* (87), *Bifidobacterium* (87), *Lactobacillus* (87), and *Staphylococcus* (87) were found.

ARGs

After treatment with ceftriaxone, one study found *Klebsiella/ Enterobacter, Citrobacter, Serratia* and *E. coli* to be resistant to cefoperazone and ceftriaxone and *Pseudomonas aeruginosa* to ceftriaxone (60). One study found increased abundances of multiple ARGs after treatment with cefotaxime, e.g., *β-lactamase (CMY-LAT-MOX), MFS efflux, ABC efflux,* and *robA* (55).

Carbapenems

The effect of carbapenems on the intestinal microbiota was investigated in three studies including 67 children (for detailed information see Supplementary Table S3) (55, 96, 105).

Diversity

One study found a reduced richness comparing before and two days after treatment with meropenem (55). Two studies did not analyse alpha or beta diversity (96, 105).

Composition

The studies reported an increased bacterial count of *Enterococcus* (96), *Proteus* (96), *Pseudomonas* (96), *Enterobacter* (96), and *Staphylococcus epidermidis* (55), and a decreased abundance of *Klebsiella* (96), *Lactobacillus* (96), and *Streptococcus* (96) comparing before and after treatment with imipenem-cilastatin, while the second study did not find changes in the abundance of different bacteria (105).

ARGs

The first study did not identify bacteria with resistance to imipenem (96), while in the second study, in one child *P. aeruginosa* resistant to imipenem was found after treatment. This child was previously also treated with aztreonam (105). One study found increased abundances of multiple ARGs after treatment with meropenem, e.g., *mecA, norA, dfrC, gyrA,* and *qacA* (55).

Macrolides

The effect of macrolides on the intestinal microbiota was investigated in 13 studies including 802 children (for detailed information see Supplementary Table S3) (38, 46, 62, 70, 71, 75, 79, 80, 87, 95, 103, 111, 112).

Diversity

Four studies found a lower alpha diversity (46, 79, 95, 103) and three a lower richness (70, 75, 80) between five days and 12–24 months after treatment with macrolides. One study reported a decrease in alpha diversity after 14 days but no further changes between 13 and 39 months after treatment with azithromycin (95). After treatment with azithromycin, one study found a difference in richness between children with ABX and controls, but no difference in alpha diversity (80) (Figure 2). Three studies analysed beta diversity and found a high beta diversity between ABX group and controls up to 14 days after treatment with azithromycin (80, 95) and a distinct composition on phylum and genus level in the ABX group up until six months after treatment with either azithromycin or clarithromycin without reporting results separately (70). One study did not detect a difference in beta diversity five days after treatment with azithromycin (46).

Composition

Compared to controls, after treatment with azithromycin increased abundances/bacterial counts of *Clostridium* (95), and *Blautia* (46), and decreased abundances/bacterial counts of Actinobacteria (95), Verrucomicrobia (80), *Betaproteobacteria* (80), *Verrucomicrobiae* (80), *Bifidobacteriales* (95), *Bifidobacteriaceae* (95), *Clostridiacea* (95), *Bifidobacterium* (95), *Anaerovibrio* (46), *Peptoniphilus* (46), *Succinivibrio* (46), *Megasphaera* (46), *Escherichia* (80), *Akkermansia* (80), *Peptostreptococcus* (80), *Campylobacter hominis* (62), *Campylobacter ureolyticus* (62), and *Campylobacter jejuni* (62) were found. Two studies did not report separate results for changes in abundances after treatment with different macrolides and found compared to controls, increased abundances of *Bacteroidetes* (70), *Alphaproteobacteria* (71), *Bacteroidales* (70), *Lactobacillales* (70), *Rikenellaceae* (70), *Subdoligranulum* (71), *Salmonella* (71), *Eggerthella* (70), *Bacteroides* (70), *Parabacteroides* (70), *Eubacterium* (70), and *Clostridium* (70), and decreased abundances of Actinobacteria (70), *Bifidobacteriales* (70), *Coriobacteriales* (70), *Clostridiales* (70), *Gemellaceae* (70), *Collinsella* (70), and *Bifidobacterium* (70, 71). Conflicting results were reported for abundances of Proteobacteria (70, 80), *Bacillales/Gemellales* (70, 71), and *Dialister* (70, 95) (Figure 3). Two studies found no difference in bacterial abundance between children with ABX and controls (38, 75). Three studies did report *p*-values for abundances (38, 79, 111). One study did not investigate bacterial abundances (103) (Figure 5).

ARGs

ARGs were investigated in two studies (70, 114). One study found increased macrolide resistance after treatment with azithromycin or clarithromycin (without separate analysis), which declined linearly until going back to baseline 6 to 12 months after ABX. The study also found that the abundance of *ermF* and *ermB* genes correlated negatively with time since the last macrolide treatment, while the abundance of the *bsh* gene correlated positively with time since the last macrolide treatment (70). One study found a higher abundance of ARGs and plasmids in the ABX group compared with controls, without performing a separate analysis for different macrolides (75).

Trimethoprim/sulfamethoxazole

The effect of trimethoprim/sulfamethoxazole on the intestinal microbiota was investigated in six studies, including 254 children (27, 36, 47, 53, 55, 79).

Diversity

Two studies detected no difference in alpha diversity between the ABX group and the control group within the first week after ABX and after seven and eight years of continuous prophylactic treatment (Figure 2) (36, 79). Another study found a longitudinal increase in alpha diversity between six weeks and six months after the ABX in the ABX group (47). Another study found a decrease in diversity two weeks after the start of prophylactic treatment compared to before treatment, which recovered within one to two months (27). Another study found a decreased richness comparing before and two days after ABX (55). One study found no difference in bacterial counts between the two groups (53). Two studies analysed beta diversity, of which one did not detect a difference in between the ABX group and controls (36) and the other one found a lower beta diversity in the ABX group compared to controls (47). Comparing before to after treatment with trimethoprim/sulfamethoxazole decreased bacterial counts of *Enterobacter* (53) and *Veillonella* (53) were found.

Composition

Comparing the ABX group to controls increased abundances of Enterobacteriales (27), *Alistipes onderdonkii* (36), *Eggerthella lenta* (36), *Clostridium bartlettii* (36). *Haemophilus parainfluenzae* (36), *Streptococcus mutans* (36), *Streptococcus parasanguinis* (36), and *Streptococcus vestibularis* (36), and decreased abundances of *Enterobacteriaceae* (36) were found after ABX. One study detected no differences in bacterial abundance after treatment with trimethoprim/sulfamethoxazole (47) and another study did not provide *p*-values for changes in abundances (79).

ARGs

ARGs were studied in one study, *dfr* and *sul* genes were found to be more abundant in the ABX group after the ABX compared to before (47).

FIGURE 5
Differences in bacterial abundance or total bacterial count between children treated with macrolides and controls. Included studies investigated azithromycin (80, 95, 103), azithromycin and clarithromycin (70) or did not separately analyse different macrolides (71). Studies which did not compare ABX group to controls and studies not providing *p*-values were excluded.

Aminoglycosides

The effect of aminoglycosides on the intestinal microbiota was investigated in three studies, including 52 children (for detailed information see Supplementary Table S3) (55, 87, 112).

Diversity

The first study found no difference in alpha diversity three days after treatment with gentamicin (55) and the second did not analyse alpha diversity (87). Both studies did not analyse beta diversity.

Composition

One study observed decreased bacterial counts of *Enterobacteriaceae* (87), *Streptococcus* (87), *Clostridium* (87), and *Lactobacillus* (87) comparing before and three to six days after treatment with gentamicin.

ARGs

One study found increased abundances of multiple ARGs after treatment with gentamicin, e.g., *evgA* and *emrK* (55).

Glycopeptides

The effect of glycopeptides on the intestinal microbiota was investigated in three studies, including 48 children (for detailed information see Supplementary Table S3) (45, 55, 84).

Diversity

One study found a lower alpha diversity seven days after treatment with vancomycin compared to before ABX, but no difference between the ABX group and controls (45). Another study found a lower richness two days after treatment with vancomycin compared to before ABX (55). One study found no difference in alpha diversity after treatment with vancomycin at 25 days of life (84).

Composition

One study found increased abundances of *Staphylococcus* and decreased abundances of *Commamonadaceae, Pseudomonas, Bifidobacterium* when comparing before and after ABX (45). Another study reported increased abundances of *Staphylococcus* (in intestinal tissue but not in stool) when comparing children treated with vancomycin to other ABX (84).

ARGs

One study found an increase in ARGs after treatment with vancomycin, e.g., *evgA, emrK* (55).

Mixed and not specified ABX

Various ABX without reporting separate results were investigated in 19 studies (24, 31–33, 44, 48, 49, 54, 59, 64–66, 72–74, 77, 86, 92, 97), while another 20 did not specify which ABX were investigated (28–30, 38, 42, 43, 50, 51, 56, 61, 67, 68, 76, 82, 98, 99, 104, 106, 109, 115). These studies included a total of 5,139 children (for detailed information see Supplementary Table S3).

Diversity

A decreased alpha diversity in ABX compared to controls was found in seven studies (29, 54, 72, 74, 77, 97, 98) and lower richness in two studies (66, 99). One study found a decrease in alpha diversity during ABX and an increase afterwards (64). One study found a lower alpha diversity in children treated for two days compared to seven days or more (44). Two studies reported that alpha diversity inversely correlated with increase in number of ABX courses (54, 74). One study found a decrease in diversity and in richness with each additional day of ABX (76) and another study found a lower diversity within the Bacteroidetes phylum in the ABX group than in controls (48). Other studies found no difference in alpha diversity between birth and hospital discharge (86), at ten days of life (43), or up to six month of life, when comparing children after ABX to controls (92). One study found no difference in alpha diversity comparing before, during, and after ABX (25). Twelve studies did not analyse alpha diversity or richness (28, 31–33, 39, 42, 49, 56, 61, 67, 73, 82, 104). Five studies found a high beta diversity with a dissimilar bacterial composition between the ABX group and controls (54, 72, 92, 98, 25). One study found a dissimilar composition in children with two days of ABX compared to seven or more days (44). One study found a dissimilar composition between neonates receiving ABX in the first week of life only and neonates receiving ABX in the first week of life plus after the first week of life (54). Samples collected in controls showed more similarity to each other than samples from the period when children were starting or stopping ABX (30). One study found no effect of ABX on beta diversity at one year of life (51). Another study found a decrease in beta diversity in neonates from week one to week three of life in the ABX group compared to controls (97).

Composition

When comparing ABX groups and controls, after ABX, increased abundances/colonisation rates/bacterial counts for Proteobacteria (28), *Gammaproteobacteria* (73),

Enterobacteriaceae (29, 54), *Veillonella* (54), *Klebsiella* (56), Escherichia/Shigella (72), *Enterobacter* (56), *Klebsiella/ Enterobacter* (31), *Sphingomonas* (97), *Acidovorax* (97), *Proteus* (42), *Bacteroides vulgatus* (74), *Bifidobacterium bifidum* (74), *Staphylococcus epidermidis* (54), *Veillonella parvula* (54), *Veillonella unspecified* (54), *Klebsiella oxytoca* (54), *Escherichia coli* (54), *Bifidobacterium breve* (54), and decreased abundances for Bacteroidetes (48, 72, 76, 92), *Clostridiales* (54), *Bifidobacteraceae* (54), *Prevotella* (54), *Bacteroides* (29, 65, 72, 77, 82), *Parabacteroides* (29), *Ruminococcus* (25), *Haemophilus* (97), *Blautia* (97), *Erysipelatoclostridium* (97), *Gemella* (97), *Rothia* (97), *Streptococcus* (97), *Clostridium perfringens* (33), *Eubacterium rectale* (98), *Akkermansia muciniphila* (80), *Lactobacillus mucosae* (80), *Bacteroides fragilis* (80), *Actinomyces odontolyticus* (74), *Bifidobacterium longum* (50, 54, 74), *Bifidobacterium bifidum* (50, 54) *Bifidobacterium lactis* (50), *Escherichia coli* (30, 31) *Escherichia coli 8711* (80), *and Escherichia coli 17709* (80) were found. Conflicting results were found for Actinobacteria (28, 72), Firmicutes (28, 72, 92), *Clostridium* (29, 65, 73, 76, 98), *Enterococcus* (54, 77, 97), *Lactobacillus* (32, 33, 61, 65, 92), *Bifidobacterium* (29, 32, 54, 72, 82), *Citrobacter* (42, 56), *Staphylococcus* (54, 97), *Clostridium difficile* (33, 67), *Clostridium butyricum* (33, 49), *Enterococcus faecialis* (31, 54), and *Bifidobacterium breve* (50, 54, 74) (Figure 6). One study found *Clostridium perfringens* abundance decreased compared to controls 3–4 weeks after ABX and increased 5–6 weeks after ABX (42). One study found a lower abundance of Bacteroides and a higher abundance of Actinobacteria, Proteobacteria, *Bacteroidetes* in children treated for more than seven days compared to these treated for two days (44). Another study found lower abundances of *Lactobacillus* and *Enterococcus* after more than seven days of treatment compared to less than seven days of treatment (68). Three studies did not compare bacterial abundances (66, 99, 104). One study, only investigating *Bifidobacterium* abundance, found no difference in abundance between the ABX group and controls (39).

ARGs

ARGs were studied in two studies, which found a higher abundance of ARGs in ABX group including resistance to ABX rarely/never used in neonates and to multidrug-resistant organisms (MDROs) (54), higher abundance of ARGs and episomally encoded genes in the ABX group (98).

Discussion

The first three years of life are pivotal for the development of the intestinal microbiota (14, 25), which, in turn, plays a crucial role in shaping the immune system. Moreover, early colonization of the intestine significantly impacts later microbial communities, so changes in this early period will have long-lasting consequences (115). A lower diversity of the intestinal microbiota has been associated with an increased risk of developing allergic diseases, type 1 diabetes, and rheumatic diseases (116–118). It is therefore concerning that all the ABX investigated in the studies in our review had profound effects on the intestinal microbiota in children associated with a decrease in alpha diversity. In some studies, this decrease was positively associated with duration of ABX. This effect was prolonged, persisting up to 12–24 months after stopping ABX for macrolides, and up to 36 months for ABX in the neonatal period.

In our review, we found that exposure to certain ABX (penicillins, penicillins plus gentamicin, cephalosporins, carbapenems, macrolides, and aminoglycosides, but not trimethoprim/sulfamethoxazole) is associated with decreased abundances of Actinobacteria (52, 70, 95), *Bifidobacteriales* (70, 95), *Bifidobacteriaceae* (52, 54, 95), and/or *Bifidobacterium* (52, 57, 70, 83, 87), and *Lactobacillus* (52, 70, 87, 88, 96). The direction of change in the abundance of *Enterobacteriaceae* depends on the ABX class but often an increase in *Enterobacteriaceae* other than *E. coli* is observed. These findings are in accordance with findings from a similar review in adults, which, after ABX, along with a decrease in alpha diversity, also found decreased abundances of *Bifidobacterium* and *Lactobacillus* and increased abundances of *Enterobacteriaceae,* other than *E. coli* (e.g., *Klebsiella, Citrobacter* and *Enterobacter*) (16). The results from our review, also align with results from a large *in vitro* study, which tested the effect of 144 ABX on 38 common human intestinal microbiota species (119). The study found that macrolides strongly inhibit the growth of most tested intestinal microbes, while beta-lactams have strain specific effects. This strain-specific effect of ABX might lead to large community composition disturbances with "killing-sensitive" strains more readily being eliminated from communities.

As mentioned above, almost all ABX are associated with a reduction in bacteria which have been identified as being beneficial. *Bifidobacterium* and *Lactobacillus* contribute to maintaining the gut barrier by producing high concentrations of short chain fatty acids (SCFA) such as acetate, propionate, and butyrate (120). However, SCFA not only serve as energy sources for the interstitial epithelium, but also have diverse effects on host physiology and immunity. In children, a decreased abundance of Actinobacteria has been associated with type 1 diabetes (121), while lower abundances of *Lactobacillaceae* and *Bifidobacteriaceae* have been associated with the development of allergic sensitization, eczema, or asthma (116). Reduced *Bifidobacterium* abundance has also been linked to childhood obesity (122, 123). Furthermore, it has been shown that peptidoglycans from *Bifidobacterium* can cross the blood-brain barrier and enhance neuronal maturation by influencing cytokine production by microglia (124). However, it's important to note that most of the studies investigating associations between variations in the intestinal microbiota composition and diseases are cross-sectional and therefore, it cannot be determined whether factors like ABX use, which may transiently decrease the abundance of different beneficial taxa, impact the development of these diseases. Nevertheless, a large meta-analysis has shown that ABX exposure in the first years of life is associated with an increased risk of developing atopic dermatitis, allergies, wheezing


Actinomycetota
Actinomycetes
Bifidobacteriales
Bifidobacteriaceae
Bifidobacterium
Micrococcales
Micrococcaceae
Rothia
Bacteroidota
Bacteroidia
Bacteroidales
Bacteroidaceae
Bacteroides
Prevotellaceae
Prevotella
Tannerellaceae
Parabacteroides
Bacillota
Clostridia
Eubacteriales
Oscillospiraceae
Ruminococcus
Clostridiaceae
Clostridium
Lachnospirales
Lachnospiraceae
Blautia
Peptostreptococcales
Peptostreptococcaceae
Clostridioides
Bacilli
Bacillales
Staphylococcaceae
Staphylococcus
Gemellaceae
Gemella
Lactobacillales
Lactobacillaceae
Lactobacillus
Enterococcaceae
Enterococcus
Streptococcaceae
Streptococcus
Erysipelotrichia
Erysipelotrichales
Coprobacillaceae
Erysipelatoclostridium
Negativicutes
Selenomonadales
Selenomonadaceae
Megamonas
Veillonellales
Veillonellaceae
Veillonella
Pseudomonadota
Gammaproteobacteria
Enterobacterales
Enterobacteriaceae
Klebsiella
Enterobacter
Citrobacter
Morganellaceae
Proteus
Pasteurellales
Pasteurellaceae
Haemophilus
Alphaproteobacteria
Sphingomonadales
Sphingomonadaceae
Sphingomonas
Betaproteobacteria
Burkholderiales
Comamonadaceae
Acidovorax
Firmicutes
Proteobacteria
Lower abundance in 1 study
Lower abundance in 2 studies
Lower abundance in > 2 studies
Higher abundance in 1 study
Higher abundance in 2 studies
Higher abundance in > 2 studies
Higher AND lower abundance found across studies

FIGURE 6

Differences in bacterial abundance or colonisation rate between ABX groups and controls of studies investigating various ABX without separate analysis of individual ABX. Included studies investigated: ampicillin, nafcillin, gentamicin, tobramycin, others (97), penicillin (plus aminoglycoside), others (92), ampicillin/sulbactam, cefotaxime (72), penicillin plus gentamicin, amoxicillin plus gentamicin, amoxicillin plus ceftazidime, others (48), ampicillin and cefotaxime, amikacin, vancomycin, others (65), ampicillin, cefotaxime, gentamicin, others (54), cephalosporin, penicillin, others (113), penicillins, macrolides, cephalosporins, others (115), beta-lactams, aminoglycosides, vancomycin, others (73), benzylpenicillin, cloxacillin, flucloxacillin, others (32), benzylpenicillin, cloxacillin, flucloxacillin, others (31), penicillin, penicillin plus gentamicin, others (33), or did not specify which ABX they investigated (28, 29, 42, 56, 61, 82, 98). Studies which did not compare ABX group to controls and studies not providing p-values were excluded.

and asthma, obesity, rheumatological and neurodevelopmental diseases (12) and it is very likely that the mechanism behind this are changes in the microbiota (125).

The exact mechanism by which different intestinal bacteria influence the developing immune system is not yet clear but dysbiosis has been associated with a pro-inflammatory state (e.g., increased T-helper 17 cells (126). A decreased abundance of *Bacteroides uniformis* has been associated with increased production of interleukin (IL)-17, which is associated with increased neutrophil extracellular trap (NET) formation (114). These web-like structures are composed of DNA, histones, and antimicrobial proteins and released by activated neutrophils in response to infection or inflammation. NETs play an important role in fighting pathogens. In critically ill patients, progressive intestinal dysbiosis characterised by a high abundance of *Enterobacteriaceae* has been associated with a shift towards immature neutrophil populations with reduced NET formation (127). In contrast, dysregulated NET formation has been associated with various inflammatory and autoimmune diseases, e.g., rheumatological diseases (128). Other studies showed that the abundance of *Klebsiella pneumoniae* and *Streptococcus mitis* in the intestine positively correlates with the amount of natural killer cells in blood, the abundance of *B. uniformis* with immunoglobulin M levels as well as the erythrocyte sedimentation rate, and the abundance *Eubacterium eligens* with IL-4 and $CD3^+CD8^+$ T cells levels (129).

Antimicrobial resistance is an increasing problem; it is estimated that by the year 2050, 10 million people will die annually because of infections with ABX-resistant bacteria (130). Therefore, another important finding in our review is the increase in ARG following ABX with all ABX classes, which persisted for as short as three weeks for some ABX, but up to four months after treatment for others. This is particularly important for ABX, such as amoxicillin and trimethoprim/sulfamethoxazole, which are often given for long-term prophylaxis. Even a transient increase in ARGs can have significant clinical implications, especially if a child develops a new infection during this period, as it may lead to infections that are harder to treat, requiring ABX with more side effects or which have broader spectrum activity, further exacerbating the problem of antimicrobial resistance.

The impact of ABX on the intestinal microbiota is multifaceted, influenced by both their spectrum of activity, and whether ABX are administered orally or intravenously. Formulation is also a factor: for example bacampicillin syrup alters bacterial abundance but tablets do not (131). ABX with a broad spectrum of activity and selective killing are the most dangerous to the intestinal microbiota and it is important to find drugs with a narrow spectrum of activity that inhibit pathogens but not intestinal commensals.

Other factors can mitigate ABX-induced dysbiosis. In infants, this includes breastfeeding and administration of pre-, pro- and postbiotics (132). Breastfeeding has been associated with an increased abundance of *Bifidobacterium, Staphylococcus,* and *Streptococcus* and lower abundances of *Enterococcus* and *Enterobacteriaceae* (133). Breast milk itself is an important source of pre-, pro- and postbiotics. A recent study showed that breastfeeding reduced the decrease in *B. infantis* which was seen after ABX and protected from ABX-associated increased asthma risk (134). The European Society for Paediatric Gastroenterology Hepatology and Nutrition recommends probiotics for the prevention of ABX-associated diarrhoea (132, 135), particularly *Saccharomyces boulardii* and *Lactobacillus rhamnosus* GG (136). In an RCT involving healthy children, the administration of *Bacillus subtilis* DE111 increased alpha diversity (137). Similarly, an observational study in preterm neonates showed an increase in alpha diversity, higher abundances of *Bifidobacterium* and *Lactobacillus*, and lower abundances of *Streptococcus* after the administration of a probiotic containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (138). These findings, which contrast with the ABX-induced microbial disturbances observed in our systematic review, suggest that probiotics might help restore gut microbiota balance after ABX. A Cochrane review including 33 RCTs concluded that the administration of probiotics led to a reduction of ABX-associated diarrhoea from 19 to 8% with a number needed to treat of nine (132). However, many important questions remain open, such as the ideal timing, dosage, duration and strain selection for probiotics, as well as the benefit of co-administration with pre- and postbiotics.

Strengths and limitations

A strength of this study is the comprehensive literature search, including children of all age groups and various ABX classes. However, the study is also subject to some limitations: First, most of the included studies were observational studies and only few RCTs were identified. Second, although most studies used longitudinal designs, the variability in follow-up periods, ranging from days to years, complicates inter-study comparisons. The timing of stool sampling is crucial, as changes in the bacterial composition of the intestinal microbiota can be transient or fluctuant. Third, a significant portion of the studies did not differentiate between various ABX or did not specify ABX. This may introduce bias into the results, as

different ABX classes have been shown to have different effects. Therefore, the results of these studies have been pooled for this review. Even within an ABX class, the spectrum of activity differs and the effect on the intestinal microbiota will therefore be different. Fourth, except for five studies, all included participants had (suspected) infections, potentially introducing infections as a confounding factor. Furthermore, the analysis encompassed diverse age groups, though the impact of ABX on the microbiota is likely most pronounced in younger children, particularly neonates or infants. Lastly, microbiota research is largely influenced by the used analysis techniques. Molecular diagnostics are influenced by DNA extraction and library preparation method, used sequencing platforms and protocols and bioinformatic pipelines and tools. Culture-based diagnostics are influenced by the choice of culture media and incubation conditions and techniques for assessing ABX resistance.

In summary, ABX have profound effects on the intestinal microbiota, with notable differences between ABX classes. The duration of ABX likely influences the magnitude of these changes. Among those studied, macrolides have the most substantial impact while trimethoprim/sulfamethoxazole has the least pronounced effect. Important remaining questions include how long ABX-induced changes in the composition of the intestinal microbiota persist and the long-term effect of transient changes on health outcomes, particularly if they are given beyond the critical period of microbiota development in the first two to three years of life. Additionally, it is crucial to investigate whether ABX-resistant strains persist in the absence of selective pressure from ABX. Another area for further research is the development of remedies which can selectively protect intestinal microbiomes.

Author contributions

JW: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. NC: Writing – review & editing. PZ: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. PZ is supported by an Ambizione Grant from the Swiss National Science Foundation (PZPGP3_193140).

Acknowledgments

Poh Chua provided valuable assistance in defining the search terms.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/falgy.2024.1458688/full#supplementary-material

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frontiers | Frontiers in Allergy

TYPE Mini Review
PUBLISHED 14 October 2024
DOI 10.3389/falgy.2024.1469426

OPEN ACCESS

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RECEIVED 23 July 2024
ACCEPTED 28 August 2024
PUBLISHED 14 October 2024

CITATION
Donald K and Finlay BB (2024) Mechanisms of microbe-mediated immune development in the context of antibiotics and asthma.
Front. Allergy 5:1469426.
doi: 10.3389/falgy.2024.1469426

Mechanisms of microbe-mediated immune development in the context of antibiotics and asthma

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The gut houses 70%–80% of the body's immune cells and represents the main point of contact between the immune system and the outside world. Immune maturation occurs largely after birth and is guided by the gut microbiota. In addition to the many human clinical studies that have identified relationships between gut microbiota composition and disease outcomes, experimental research has demonstrated a plethora of mechanisms by which specific microbes and microbial metabolites train the developing immune system. The healthy maturation of the gut microbiota has been well-characterized and discreet stages marked by changes in abundance of specific microbes have been identified. Building on Chapter 8, which discusses experimental models used to study the relationship between the gut microbiota and asthma, the present review aims to dive deeper into the specific microbes and metabolites that drive key processes in immune development. The implications of microbiota maturation patterns in the context of asthma and allergies, as well as the effects of antibiotics on microbe-immune crosstalk, will also be discussed.

KEYWORDS

asthma, allergies, microbial metabolites, microbe-mediated immune imprinting, antibiotics

1 Introduction

1.1 The infant gut microbiota: general patterns in colonization

The gut microbiota is highly dynamic during the neonatal period, and does not reach stability until 3–5 years of age (1). Although there is a basic trajectory of colonization common to most infants, numerous environmental and host factors shape the progression of microbiota establishment. At birth, the infant gut is colonized by aerobic and facultative anaerobic bacteria largely belonging to the Proteobacteria phylum, including *Enterobacteriaceae* species such as *Escherichia coli* and *Klebsiella*. These microbes consume oxygen and establish an anaerobic niche within the gut, enabling colonization of primarily Actinobacteria (including *Bifidobacteria* species), along with members of the Firmicutes and Bacteroides phyla over the first months of life (2, 3). Birth mode is the major determinant of microbiota composition in the first weeks of life (4).

The "Bifidobacterium peak", a period during which *Bifidobacteria* species dominate in the infant gut, is established as aerobic bacteria rapidly drop in numbers, and persists for the first few months of life. Feeding practice replaces birth mode as the major determinant

of gut microbiota composition. Breastmilk contains human milk oligosaccharides (HMOs), complex prebiotic sugars which directly promote *Bifidobacteria* species and cannot be digested by the infant (5). Breastfed infants thus display a stronger and more persistent Bifidobacterium peak than formula-fed infants. Around the time of solid food introduction (4–6 months), Bifidobacteria species decline and are replaced gradually by Clostridia and some Bacteroides species (6, 7).

The maturation patterns of the infant gut microbiota have been well-characterized through mathematical models, which are designed predict age based on gut microbiota composition (8, 9). Using these models, researchers have found that slow diversification and a strong and persistent Bifidobacterium peak are the hallmarks of healthy microbiota maturation, and that premature diversification is associated with poor health outcomes (7, 10).

1.2 Infant immune development: key features

The period between birth and 3–5 years of age represents a critical period of microbe-mediated immune imprinting that affect life-long systemic health (Figure 1) (11, 12). However, some compartments of the immune system develop *in utero*, and may be affected by the maternal microbiota. Although the placenta is devoid of bacteria, cytokines and bacterial metabolites can cross the placenta, enabling cross-talk between maternal immune responses to the gut microbiota and fetal development (13). De Agüero et al. were able to transiently colonize otherwise germ-free pregnant mice with *Escherichia coli*, such that pups would only experience the effects of the microbe *in utero* (14). They found that pups born to transiently colonized dams displayed increases in several immune compartments important for recognizing and responding to the colonizing microbiota, and that transfer of maternal antibodies across the placenta was responsible for this. This indicates that the influence of the microbiota on immune maturation begins even before birth.

The neonatal immune system is uniquely suited to face the influx of antigenic stimulation that occurs at birth. Newborn T cell responses that are prone toward regulatory and Th2 phenotypes, limiting Th1-mediated inflammation that would typically be induced by foreign bacterial antigens (15). Newborns also display reduced blood neutrophil and monocyte levels, and impaired Toll-like receptor-mediated microbial recognition (16–18). Regulatory cytokines IL-10 and IL-27, which limit inflammation, are elevated in infant blood. Although B cell and dendritic cell (DC) responses converge with maternal phenotypes by 3 months, T cell responses take longer to fully develop, and inflammatory Th1 cell levels increase only after 1 month of age (19). These results come from studies of human peripheral and cord blood samples taken in the first moments or days of life, and illustrate that neonatal systemic immune system is poised to tolerate the colonization and establishment of the gut microbiota.

The gut harbors 70%–80% of the body's immune cells, and analyses of human blood fail to capture immune development occurring at this important mucosal barrier (20). Due to the difficulty in obtaining intestinal tissue samples from human infants, most of our knowledge of gut mucosal immune development comes from animal studies. While the specific timing and order of immune maturation differ between species, the role of microbes and the general patterns of gut development are similar. In both mouse and human neonates, mucous secretion, cell proliferation, antimicrobial peptide production, and immune cell numbers are reduced (21). Over the course of the first few weeks in mice and months in humans, microbial expansion drives the development of an intact mucosal barrier. The interactions that occur during this period not only affect local processes such as oral tolerance and defense against intestinal pathogens and pathobionts, but also systemic immunity and allergy susceptibility (22). The following sections will highlight the major microbes and metabolites that affect specific immune cell populations at both the mucosal and systemic level, with a focus specifically on T cell populations that affect asthma and allergy outcomes. The progression of infant gut microbiota and T cell development is summarized in Figure 1.

2 Short chain fatty acids

Short chain fatty acids (SCFAs) are the most well-characterized bacterial products that affect systemic immunity. Acetate, propionate, and butyrate, the most common SCFAs, are produced in the breakdown of prebiotics such as dietary fiber (23). They are taken up by colonocytes and released into circulation to affect a variety of cell types. They primarily bind to either G protein coupled receptors, affecting intracellular pathways, or histone deacetylases, turning on gene expression (24). Among countless other functions throughout the body, SCFAs promote regulatory and anti-inflammatory immune responses.

Acetate alone rescues the altered thymic T cell development observed in germ-free mice (25), and is anti-inflammatory in cultured human-derived organoids (26). All 3 major SCFAs can promote IL-10 production and regulatory T cell (Treg) phenotypes, contributing to immune tolerance and limiting inflammation. This is achieved through direct binding to G protein coupled receptors, affecting downstream pathways in immune cells or by direct inhibition of histone deacetylases that act on *Foxp3*, the gene that encodes the Treg defining transcription factor in the gut (27). Butyrate can also promote gut barrier integrity and limit gut permeability by inducing tight junction expression and mucous production (28).

During the first few months of life, *Bifidobacteria* species, which make up the majority of the gut microbiota in breastfed infants, produce high levels of acetate in the breakdown of HMOs (Figure 1) (29). Changes in diet and environmental exposure, along with cross-feeding of acetate, promote the slow rise of butyrate and propionate producing bacteria mainly belonging to the Firmicutes phylum. While some *Prevotellaceae* species in the Bacteroides phylum produce SCFAs, the *Lachnospiraceae* family includes the most butyrate and propionate producers (30). Dietary fiber replaces HMOs as the

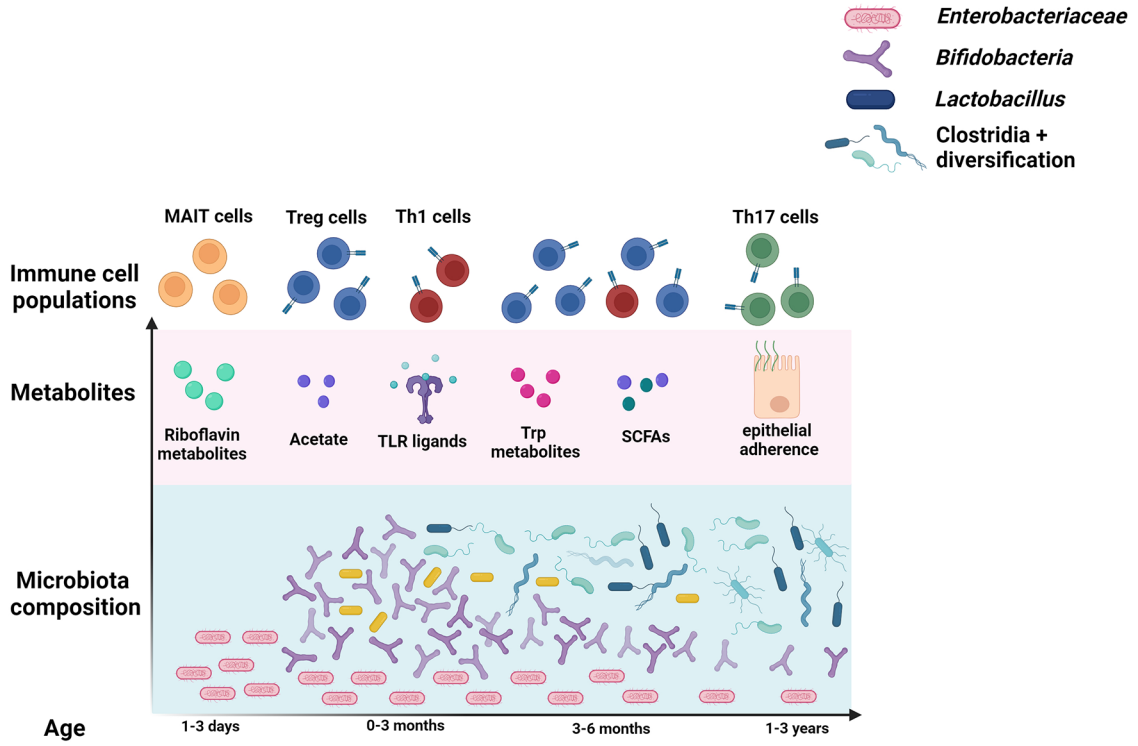

FIGURE 1
Microbe-mediated immune system imprinting in early life.

primary substrate for SCFA production, contributing to a stable and healthy gut community.

The anti-inflammatory effects of SCFAs have been shown to directly limit allergic phenotypes in animal studies. Feeding mice a high fiber diet promotes SCFA producers and limits inflammatory responses in asthma models by increasing Treg responses (31, 32). The weaning reaction to the colonizing microbiota, discussed in Chapter 8, involves SCFA-induced Treg development which protects against later development of Th2-mediated disease (33). Importantly, microbial exposure after weaning is not sufficient to rescue the phenotype, implicating the early-life window as an essential period of SCFA-mediated immune imprinting. SCFAs can even cross the placenta, and acetate has been shown to promote Treg development in the fetal lung and protect against later development of asthma when produced at high levels by the maternal microbiota (34).

The role of SCFAs in allergy protection is also supported by human data. Levels of acetate-producing Bifidobacteria before 6 months are inversely correlated with allergic outcomes (35–37). The abundance of several SCFA producing *Lachnospiraceae* species are also thought to be protective against allergies (38). Levels of butyrate producers are reduced in 1 year old infants that develop allergies (37), and plasma levels of SCFAs are reduced in infants who go on to develop atopic disease (39). Additionally, infants born to mothers who carry the acetate-producer *Prevotella copri* during pregnancy were found to be significantly less likely to develop food allergies (40). This relationship did not depend on whether the infant also carried *P. copri*, potentially implicating trans-placental acetate in the protective effect.

3 Microbial stimulation of regulatory pathways

SCFAs are not the only anti-inflammatory metabolites produced by bacteria in the gut. Indole-3-lactic acid, produced by *Bifidobacterium infantis* in tryptophan catabolism, promotes expression of the negative regulator galectin-1 in Th2 and Th17 cells, contributing to tolerance and limiting inflammation in human infants (41). This was cleverly demonstrated by exposing naïve CD4 + T cells to fecal water extracted from infants supplemented with a *B. infantis* probiotic. Other tryptophan metabolites, produced by a variety of bacteria, including *Lactobacillus reuteri*, also promote Treg responses by binding to Aryl Hydrocarbon receptors (AhR) expressed by intestinal epithelial and immune cells (42, 43).

As mentioned above, the neonatal immune system is alternatively programmed compared to that of adults. The alterations have been described in detail previously, but can be generally summarized as a proneness toward regulatory responses (7). Thus, some antigens that typically induce inflammation actually drive anti-inflammatory responses in infants. For example, newborn DCs produce the regulatory cytokine IL10 in response to the endotoxin LPS (16). Between shifts in the microbiota and the altered immune state of infants, there are

numerous mechanisms in place to prioritize Treg development, and for good evolutionary reason: Treg levels seem to be imprinted in early life, as indicated by the "weaning reaction" and the fact that germ-free mice lack intestinal Tregs, a phenotype that can only be restored by colonization during the neonatal period (44). Therefore, sufficient host-microbe interactions specifically during the first months of life are vital to developing tolerance and a strong Treg pool.

4 Microbial stimulation of inflammatory pathways

One of the original theories to explain the hygiene hypothesis was the Th1/Th2 paradigm: the idea that Th1 cells and Th2 cells reciprocally regulate each other, and that sufficient stimulation of Th1 responses was required to dampen Th2 responses involved in allergy (45, 46). Although cytokines produced by Th1 and Th2 cells do limit one another, T cells display a high level of plasticity, and alternative T cell types have emerged. Thus, the idea that the adaptive immune system exists simply as a "balance" between Th1 and Th2 cells has been largely debunked (47). However, there is ample evidence that in addition to promoting regulatory and anti-inflammatory responses, the gut microbiota must be sufficiently diverse and immunostimulatory in order to favor non-Th2 responses and promote proper immune development.

Endotoxin, a pro-inflammatory molecule released by gram-negative bacteria, is a potent stimulator of Th1 responses. In addition to promoting Treg responses, as mentioned above, endotoxin contributes to a slow maturation of Th1 responses (48). As the gut epithelium matures and becomes more proliferative, intracellular endotoxin levels shift, which slowly trains Th1 cells to respond appropriately. Oral endotoxin was also shown to dampen Th2-mediated inflammation in a murine asthma model, likely by limiting Th2-skewed DC recruitment to the lung (49, 50). Polysaccharide A (PSA), produced by *Bacteroides fragilis,* is taken up by intestinal DCs and carried to lymphoid organs to induce Th1 cells, limiting Th2 inflammation (51). Strains of *Lactobacillus*, commonly implemented as probiotics, also promote Th1-inducing cytokines by binding and stimulating innate Toll-like receptors expressed by intestinal epithelial cells (52). This has been shown to ameliorate inflammation in the OVA model of allergic asthma (53).

More recently, Th17 cells have emerged as an abundant and important T cell type particularly in the gut. Th17 cells promote barrier integrity, mucous production, and pathogen responses (54). These cells are completely lacking in GF mice, a phenotype that can be rescued by transplantation of a diverse microbiota or colonization with specific tissue-adherent microbes (44). Th17 cell responses in humans are also likely stimulated by microbes that adhere closely to the epithelial lining, rather than production of a specific metabolite (55). Although Th17 cells are protective in the context of pathogens and oral tolerance, overstimulation of Th17 pathways can contribute to allergic asthma phenotypes in humans and mouse models (56–58). A balance between Th17 and Treg pathways is thus essential for protecting against both pathogens and allergies.

Mucosal-associated invariant T (MAIT) cells, a type of innate-like lymphocyte that develop during the neonatal period, have also recently been recognized as important players in the microbiota-immune axis and asthma. Similar to Th17 cells, MAIT cells contribute to inflammation and pathogen resistance, but are thought to actually limit Th2 responses and asthma (59, 60). MAIT cells are missing in GF mice, a phenotype that can only be rescued during early life by colonization with *Enterobacteriaceae* capable of metabolizing riboflavin into MAIT-inducing antigens (61). As mentioned above, *Enterobacteriaceae* are the first colonizers of human infants, and the relationship between this family and MAIT cells may provide an evolutionary reason for this (62).

5 Antibiotics and mechanisms of microbe-immune crosstalk

There is extensive evidence that a loss of SCFAs and Tregs partially mediate the detrimental immunological effects of early life antibiotic exposure. In mice, vancomycin-induced dysbiosis in early life led to a reduction in butyrate-producing Clostridia and more severe allergic lung inflammation (63, 64). SCFA supplementation was sufficient to rescue the antibiotics-associated allergic phenotype. Cefoperazone treatment also significantly reduced SCFA levels in mice (65). Antibiotics also completely disrupt the weaning reaction, permanently reducing the levels of allergy-protective Tregs (33). In humans, antibiotic treatment disrupts the Bifidobacterium peak permanently, and transiently alters *Lachnospiraceae* levels, both of which are important contributors of SCFAs in infancy (66).

In addition to weakening the Bifidobacterium peak, antibiotics limit Bacteroides species and overall diversity in infants, which could limit Th1 induction by the microbiota and contribute to Th2-skewing and allergies (67). Recovery from antibiotics involves an early emergence of Clostridia species, and a faster diversification of the gut microbiota. While Clostridia have beneficial roles in immune activation, through SCFA production and Treg induction, some members of this class promote Th17 responses (66). Therefore, premature Clostridia colonization and general diversification in replacement of the Bifidobacterium peak may promote early and elevated Th17 responses and limit Treg induction by *Bifidobacteria*, contributing to allergic disease susceptibility. Antibiotics have also been shown to limit MAIT cell development by targeting riboflavin metabolizing microbes (68). As expected, this altered phenotype was specific to antibiotic exposure during the early-life period.

6 Conclusions

As communicated in this review, early life is a key period of immune maturation that is guided by the gut microbiota. The immune system of infants is uniquely prepared to face and respond to microbial stimulation, and the timing of antigenic exposure shapes the delicate balance of immune populations that affect life-long systemic health. There are several key stages of gut microbiota maturation that occur alongside mucosal and immune

development: Initial colonization with *Enterobacteriaceae* species contributes to MAIT cell development, a strong and prolonged "Bifidobacterium" peak drives Treg development through SCFAs and other metabolites, and a slow diversification and emergence of new SCFA producers promote barrier integrity, appropriately trained Th1 responses, and continued tolerance. Antibiotic administration during infancy significantly disrupts many of these key processes, and the studies described above demonstrate some of the many mechanistic explanations for the long-term detrimental effects of antibiotics on host health and allergic disease susceptibility.

Microbiota composition is summarized over the course of the first few years of life in blue. *Enterobcateriaceae* species seed the intestine first. The "bifidobacteria peak" occurs shortly after, and is defined by the breastfeeding period. Lactobacillus are also more abundant during the first months of life. Clostridia levels rise next, and overall diversity increases over the course of the first few years of life. The most well-studied metabolites produced by these microbes, and the T cell types they primarily stimulate, are displayed above the microbes that generally produce them.

Author contributions

KD: Writing – original draft, Writing – review & editing. BF: Funding acquisition, Supervision, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. KD is supported by the Four Year Fellowship Tuition Award, President's Academic Excellence Initiative PhD Award and International Tuition Award at the University of British Columbia.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Public Health

TYPE Mini Review
PUBLISHED 23 October 2024
DOI 10.3389/fpubh.2024.1455503

OPEN ACCESS

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RECEIVED 27 June 2024
ACCEPTED 02 October 2024
PUBLISHED 23 October 2024

CITATION
Dai DL, Petersen C and Turvey SE (2024) Reduce, reinforce, and replenish: safeguarding the early-life microbiota to reduce intergenerational health disparities. *Front. Public Health* 12:1455503. doi: 10.3389/fpubh.2024.1455503

Reduce, reinforce, and replenish: safeguarding the early-life microbiota to reduce intergenerational health disparities

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Socioeconomic (SE) disparity and health inequity are closely intertwined and associated with cross-generational increases in the rates of multiple chronic non-communicable diseases (NCDs) in North America and beyond. Coinciding with this social trend is an observed loss of biodiversity within the community of colonizing microbes that live in and on our bodies. Researchers have rightfully pointed to the microbiota as a key modifiable factor with the potential to ease existing health inequities. Although a number of studies have connected the adult microbiome to socioeconomic determinants and health outcomes, few studies have investigated the role of the infant microbiome in perpetuating these outcomes across generations. It is an essential and important question as the infant microbiota is highly sensitive to external forces, and observed shifts during this critical window often portend long-term outcomes of health and disease. While this is often studied in the context of direct modulators, such as delivery mode, family size, antibiotic exposure, and breastfeeding, many of these factors are tied to underlying socioeconomic and/or cross-generational factors. Exploring cross-generational socioeconomic and health inequities through the lens of the infant microbiome may provide valuable avenues to break these intergenerational cycles. In this review, we will focus on the impact of social inequality in infant microbiome development and discuss the benefits of prioritizing and restoring early-life microbiota maturation for reducing intergenerational health disparities.

KEYWORDS

socioeconomic status, health disparity, SES inequity, early-life exposures, intergenerational factors, microbiota

Introduction

Socioeconomic disparities are correlated with significant health inequities, as a lower socioeconomic status (SES) increases the likelihood of NCDs, such as chronic respiratory diseases, cardiometabolic diseases, oral diseases, and mental illnesses (1–5). Although researchers have identified environmental mediators, the mechanisms through which SE inequity becomes physiologically embedded remain unclear, especially regarding cross-generational health disparities that can be observed at a very young age (6–8). The observation that children from lower SES families are more likely to experience risk factors for NCDs such as obesity, cardiovascular risk, and behavioral difficulties, is alarming, as many of these diseases are associated with comorbidities that can significantly alter their lifelong health trajectories (1, 8). Thus, research into the biological underpinnings linking lower SES to greater

disease risk, with a specific focus on perinatal and early-life risk factors, is increasingly needed.

Our microbiota is a significant factor bridging environmental exposures with host physiology or the microbial community that lives in/on our bodies. In this review, we examine the gut microbiota, which has the greatest bacterial abundance and diversity in the body (9). Immediately after birth, the gut microbiota starts to assemble alongside infant development, eventually forming a stable community. During this early stage, the developing microbiota is highly sensitive to external influences, with changes impacting long-term immune regulation, metabolism, and behavior (10–13). Therefore, infancy is a critical period of microbial and childhood development that has far-reaching impacts on later childhood health and disease.

Interest surrounding the impact of SES on the infant and childhood gut microbiota has grown in recent years, and many factors known to disrupt infant gut microbial communities (e.g., elevated cesarean-sections (C-sections), difficulty maintaining breastfeeding, reduced access to fresh foods, increased antibiotic exposure, and reduced proximity to greenspace) are also encountered by SES-disadvantaged populations and communities (14–19). As such, the role the microbiota plays in observed SES-mediated childhood health inequities is gradually gaining recognition. In this review, we will highlight findings linking SES-associated factors to the infant's gut microbiota (Table 1). We will also explore accessible strategies for safeguarding and restoring the early-life microbiota in our society, with the goal of reducing health disparities for future generations (Figure 1).

Infant microbiome development

Before exploring the links between social inequity and the infant microbiome, it is important to discuss our current understanding of microbiota development. The primary seeding point for the gut microbiota is at birth, which is significantly influenced by the delivery mode, maternal microbiota, and maternally-derived metabolites and immune components (20–25). Initial colonization is primarily dominated by aerobes and facultative anaerobes, such as *Staphylococcus* and γ-Proteobacteria, which gradually reduce oxygen to support anaerobic colonizers, such as *Bifidobacterium*, *Bacteroides*, and multiple Clostridia genera (26). Within the first few months of life, species diversity is limited and composition is primarily dictated by type of milk diet (27). Later, as infants experience more varied environments and diets, their microbiota diversity increases. As such, prior to reaching a stable community, the microbiota matures in the first 1–3 years through patterned temporal shifts in species abundances that can be commonly detected across studies (11, 28, 29). Supporting the importance of this maturation process, microbiota disruptions during infancy—but not later—are broadly associated with a higher likelihood of allergic disease, obesity, and behavioral disorders (11, 12, 29, 30). Therefore, prioritizing normative microbiota maturation by limiting disruptions or implementing restorative interventions during this sensitive window can yield lifelong health benefits.

Potential pathways for social inequity to influence the infant microbiome

Effects of early-life exposures

Delivery mode

The mode of delivery is a primary driver of the initial compositional variance in the infant gut microbiota. However, multiple independent studies within developed nations have shown that women from lower SE backgrounds have a higher likelihood of undergoing C-section deliveries (31–34). Compared to infants born vaginally, infants born via C-sections harbor fewer maternal bacterial strains, display reduced diversity, and tend to have fewer *Bacteroides* and *Bifidobacterium*; and these alterations are independent of additional exposures, such as antibiotic use during delivery and a reduced ability to initiate breastfeeding (24, 25, 27, 35–40). However, the impact of this on later health is still unclear. Although a C-section delivery within some studies has been implicated in an elevated risk of childhood obesity, asthma and immune disorders (41–43), others have not observed any correlation (44, 45). This likely underscores a compounding effect of risk factors and implies that the negative outcomes associated with C-section deliveries could be outweighed by subsequent early-life experiences.

Breastfeeding

Immediately after birth, the diverse array of human milk components is crucial for shaping the infant's gut microbiota and supporting infant development (36, 39, 46, 47). These include secretory IgA (sIgA) (48), the milk microbiota, which serves as a significant seeding source (25, 49), and metabolites such as human milk oligosaccharides (HMOs), which serves as the third most abundant milk metabolite yet primarily serve to nourish 'infant-type' bacteria (50). As a constant force on the infant microbiome, breastfeeding can mitigate disruptions caused by both C-sections and antibiotic exposure (51–53). However, lower-income families often report difficulty initiating breastfeeding and shorter overall breastfeeding durations (54, 55). This has the potential to profoundly impact the infant microbiota, as formula-fed babies experience premature microbiota maturation, reduced abundance of *Bifidobacterium*, and more opportunistic pathogens compared to their breastfed counterparts (24, 27, 36, 37, 39, 52, 56, 57). These differences are potentially associated with child health consequences, as evidenced by a large, nationally representative longitudinal study in the United States ($n = 8{,}030$ participants), which identified infant feeding practices as a significant mediator between SES and early childhood obesity (58). Thus, greater support is needed for mothers and families who would otherwise breastfeed their infants, but are unable to do so.

Antibiotics

Antibiotics are a potent medical intervention that reduces or depletes pathogenic microbes and has considerably reduced human mortality since their discovery. However, antibiotics, especially broad-spectrum ones, commonly target both pathogenic and beneficial commensal bacteria indiscriminately; and extensive evidence exists for their off-target disruption of infant gut microbiota homeostasis. The detrimental effects include a reduction in species diversity, an enrichment of antimicrobial resistance genes (ARGs), the loss of

TABLE 1 Effect of SES-associated factors on infant gut microbiota.

Early life and cross-generational exposures		Associated change in infant microbiota			Reference
Category	Factor	Diversity	Maturation	Composition	
Delivery mode	C-section	↓	↓	↓***Bacteroides, Bifidobacterium, Parabacteroides, Escherichia***[C] ↑**Clostridiales**, **Enterobacteriaceae**[C] **Fewer maternal microbes**[C]	(24, 25, 27, 35–39)
Breastfeeding	Human milk fed	↓	↓	↑***Bifidobacterium, Lactobacillus***[C] ↓**Clostridiales**, **Enterobacteriaceae**, **Staphyloccoccaceae**[C] **Cessation of BF drives the maturation of the infant gut, as marked by the phylum Firmicutes**[C]	(24, 27, 36, 37, 39, 52, 56, 57)
Antibiotics	Amoxicillin, penicillins, combination antibiotics, etc.	↓	↓	↓***Bifidobacterium***, **Clostridiales**, *Bacteroides fragilis*[C] ↑ **γ-Proteobacteria**, Enterobacteriaceae, **ARGs**[C]	(35, 37, 38, 52, 59–61)
	Penicillin, vancomycin or combination	↓	n/a	↓Bacteroidetes[M] ↑Firmicutes[M]	(62, 63)
	Streptomycin	NS	n/a	↑Bacteroidetes (Porphyromonadaceae and Bacteroidaceae)[M] ↓Clostridiales[M]	
Malnutrition	Kwashiorkor/severe acute malnutrition	↓	↓	↓***Methanobrevibacter smithii***, ***Faecalibacterium prausnitzii***, ***Bifidobacterium longum*** and ***Lactobacillus*** *mucosae*[C] ↑***Streptococcus gallolyticus***, **Proteobacteria** and Fusobacteria, **Bacteroidetes**, ***Desulfovibrio*** genus and Campylobacterales order[C] ↑*B. wadsworthia* (related to ***Desulfovibrio***) and members of the order Clostridiales[C&M]	(78, 81–84)
	Malnourished diet (low protein & fat) vs. control	↑	n/a	↑Bacteroidetes, Proteobacteria[M] ↓Lactobacillaceae[M]	(79)
	Low micronutrient diet (low vitamins, zinc and iron) vs. control	↑	n/a	↓Firmicutes and Erysipelotrichaceae[M] ↑Proteobacteria and Enterobacteriaceae[M]	(80)
Environment	Older sibling	↑	↑	1 m: ↑ ***Bifidobacterium***, *Hungatella, Pediococcus* and ↓ *Clostridium*[C] 6 m–1 y: ↓ *Escherichia/Shigella*, other Enterobacteriaceae, *Veillonella*, and ↑*Prevotella*, *Eisenbergiella*[C]	(27, 37, 56, 93)
	Pets (dog, cat)	NS	↑	↑*Ruminococcus, Oscillospira*[C] ↓Streptococcaceae[C]	(27, 94)
	Farm or farm-like	NS	n/a	↑Bifidobacteriaceae *(B. infantis)*, Clostridiaceae, Aerococcaceae[C]	(95)
	Air pollution (PM_{10}, $PM_{2.5}$, NO_2)	NS	n/a	↑*Actinomyces* (Actinobacteria), *Clostridium*, *Enterococcus*, *Eubacterium* (Firmicutes), and *Haemophilus* (Proteobacteria) ↓*Alistipes*, *Phascolarctobacterium* (Proteobacteria)[C]	(96)

(Continued)

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TABLE 1 (Continued)

Early life and cross-generational exposures		Associated change in infant microbiota			Reference
Category	Factor	Diversity	Maturation	Composition	
Maternal diet	Diet intake based on FFQ	n/a	n/a	aMED score: ↑Clostridiaceae spp. and ↓*Bacteroides uniformis, Escherichia coli, [Ruminococcus] gnavus*[C] Fruit intake: ↑Clostridiaceae and ↓*Bifidobacterium*[C] Fish intake: ↑*Streptococcus agalactiae* and ↓*Bacteroides uniformis*[c] Dairy intake: ↑*Clostridium neonatale*, *C. butyricum*, *Staphylococcus* spp. and ↓*Lachnospiraceae* spp.[C]	(40)
	High fat intake vs. control (DSQ)	n/a	n/a	↑*Enterococcus* and ↓*Bacteroides*[C]	(104)
	Low-MAC	↓	n/a	↓Bacteroidales[M]	(105)
Prenatal antibiotics	Ampicillin, penicillin	↓	n/a	↓***Bifidobacterium***, ***Bacteroides***, *Blautia*, *Roseburia*, *Ruminococcus*, *Streptococcus* ↑**Proteobacteria**, *Escherichia*, *Oscillospora*, *Pseudobacter*, *Veillonella dispar*, ARGs[C]	(114, 115)
Prenatal distress	OASIS, PSS, PHQ	↓	n/a	↓*Bifidobacterium dentium*, *Bifidobacterium longum*, *Streptococcus salivarius*, *Lactobacillus rhamnosus*[C]	(120)
	EPDS, SCL, PRAQ	NS	n/a	↓*Akkermansia, Lactobacillus*[C] ↑γ-Proteobacteria[C]	(121)
	Receive stress vs. control	↓	n/a	↓*Lactobacillus*, *Streptococcus*[M]	(122)
	Stress & delivery mode	NS	n/a	↓*E. coli, Streptococcus acidominimus, Streptococcus thoraltensis DSM12221, Lactobacillus murinus ASF361* and ↑Peptococcaceae[M]	(123)
Maternal smoke exposure	Prenatal and postnatal smoking	↑	n/a	1 m: ↑*Ruminococcus* and *Akkermansia*[C] 3 m: ↑**Firmicutes** richness and diversity[C] 6 m: ↑*Bacteroides* and *Staphylococcus*[C]	(128, 129)

This table describes how early-life exposures and intergenerational transmission factors affect the infant gut microbiome. Results with multiple pieces of evidence are highlighted in bold. NS, not significant; n/a, not available; C, clinical; M, mouse; ARG, antimicrobial resistance genes; aMED, alternative Mediterranean diet; FFQ, food frequency questionnaire; DSQ, Dietary Screener Questionnaire; MAC, microbiota-accessible carbohydrates (dietary fiber); OASIS, Overall Anxiety Severity and Impairment Scale; PHQ, Patient Health Questionnaire; PSS, Perceived Stress Scale; EPDS, Edinburgh Postnatal Depression Scale; SCL-90, Symptom Checklist-90; PRAQ-R, The 10-item Pregnancy-Related Anxiety Questionnaire–Revised.

10.3389/fpubh.2024.1455503

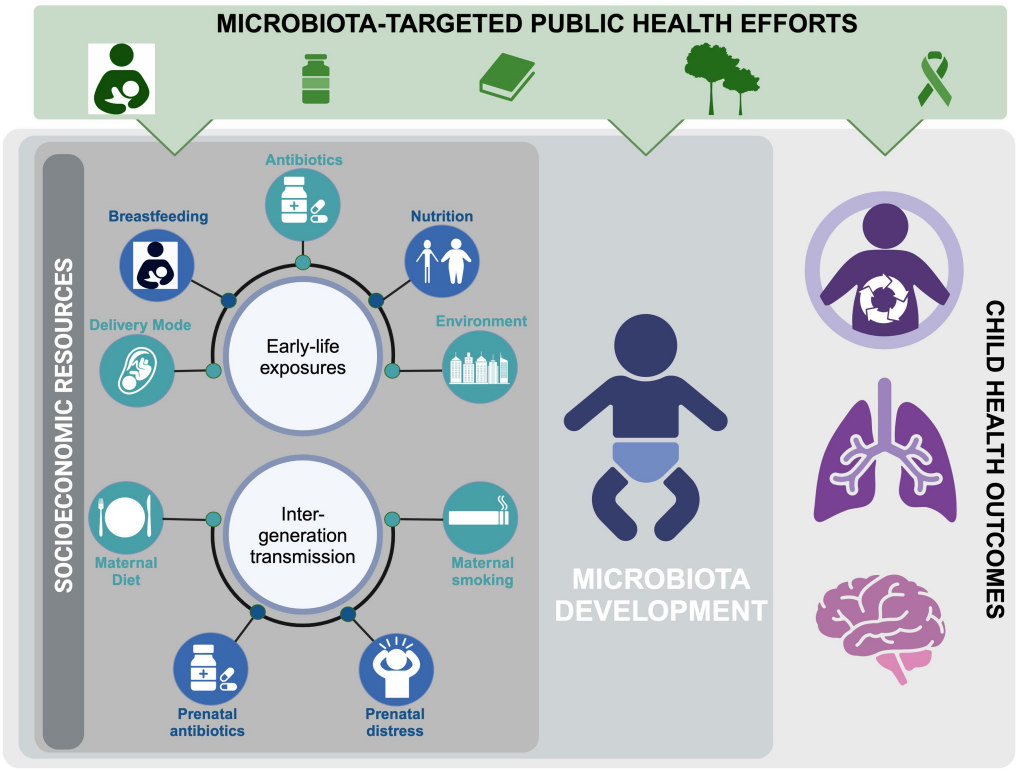

FIGURE 1
Factors influencing infant microbiome development, focusing on social inequality and potential public interventions to address disparities in child health.

beneficial bystanders such as *Bifidobacterium* and Clostridia members, and elevated γ-Proteobacteria during recovery (35, 37, 38, 52, 59–61). In addition, a number of animal studies provide evidence of the causal impact of early-life antibiotic-induced microbiota disruptions on long-term host health (62, 63). Infancy is particularly sensitive to the disruptions of early-life antibiotic exposure, which is widely associated with subsequent risk of inflammatory bowel disease, atopy, asthma, and obesity (61, 64–68). Despite being intrinsically linked to medical care access, lower SES in wealthy countries is paradoxically associated with higher early-life antibiotic exposure (69–71). Indeed, a large US study found that children from lower SES families, particularly in high-poverty areas, received more antibiotics in the first month of life despite receiving fewer antibiotic prescriptions over their lifetime (72). More alarming trends are seen from children in low- and middle-income countries, who are prescribed an average of 25 antibiotic prescriptions during their first 5 years of life. This is a remarkable amount considering that two antibiotic prescriptions per year are considered excessive in many high-income countries (73). Antibiotic stewardship initiatives, which primarily aim at reducing the spread of antimicrobial resistance, may therefore possess the additional benefit of preserving the healthy infant microbiota (60, 71, 74, 75).

Malnutrition

Malnutrition can be characterized by the over- or under-bioavailability of both macro- and micronutrients, and is commonly observed in low SES and minoritized populations (2, 76, 77). Due to its capacity to regulate nutritional harvest, the gut microbiota is an important nexus between diet and health outcomes (30). This is evidenced by a longitudinal study of Malawian twins, which found that poorly matured gut microbiota was associated with malnutrition and the causal relationship between microbiota and malnutrition is supported by multiple independent mouse studies (78–80). Malnutrition is associated with reduced obligate anaerobic species and an increase in potentially pathogenic microbes in the infant gut microbiome (78, 81–84). Furthermore, this phenomenon is widespread, as multiple studies in low-SES countries have consistently demonstrated decreased bacterial diversity in malnourished children, reduced beneficial microbes and increased pathogen enrichment (82, 84, 85). Recent randomized clinical trials have established that microbiota-directed foods, but not caloric intake alone, successfully support growth recovery in malnourished children (86, 87). These findings confirm the microbiota's role in malnutrition and emphasize the need for microbiota-informed interventions in its management.

Environment

The environment to which humans are exposed throughout their lives is a complex and important determinant of microbiota composition, with broad implications for NCDs (88–92). The infant microbiota is particularly reflective of its surroundings, with exposures from air pollution, older siblings, pets, and farms all linked to differences in composition (27, 37, 56, 93–96). Furthermore, these have enduring impacts on both the microbiota community and our health. For instance, a comprehensive Dutch study found that childhood living environments were significantly associated with adult microbiota composition despite only a weak association between childhood and adult urbanicity (97). Similarly, research has shown

that children who lived on farms or near farm-like environments during the perinatal period had a lower risk of asthma later in life (98, 99). Given that an increase in SES in urban environments is positively linked to surrounding greenspace and biodiversity, and that SES-disadvantaged neighborhoods exhibit measurably reduced microbiota diversity (92), efforts to 'rewild' our cities and expanding public access to greenspaces could be incredibly effective ways to facilitate these vital health-promoting microbial exposures.

Intergenerational transmission

Maternal diet

The quality of the maternal diet is heavily influenced by the resources afforded by SES (100–102). Not only do mothers' dietary patterns influence their own microbiota, they are also determinants of health outcomes during and after pregnancy (102, 103). As such, maternal diet can significantly influence the infant microbiota via impacting breastfeeding outcomes, altering breastmilk components, or influencing maternal microbes that seed the infant (40, 104). Importantly, these effects can persist or even compound over generations (105). The exact impacts that maternal diet has on health outcomes of offspring are best measured in well controlled animal models. For instance, after researchers in China found that maternal obesity affected child neurodevelopment, they were able to mirror the phenomenon in mice by maternal fecal microbial transplantation (FMT) and reverse the phenotype by feeding the dams high-fiber diets (106). Similarly, a maternal low-fiber diet in mice predisposed offspring to severe lower respiratory tract infections and asthma, and milk microbes from dams fed a high-fiber diet were able to rescue this effect (107). Therefore, prioritizing access to nutritiously complete maternal diets may mitigate detrimental cross-generational impacts of low SES on infant and child health.

Prenatal antibiotics

The relationship between SES and prenatal antibiotic use remains unclear, with results being largely dependent on the specific SES measures, study cohort demographics, and economic status of the origin country (108–110). However, we mention it because, despite birth being a crucial seeding point for the microbiota, the rates of antibiotic prescription during pregnancy and birth are markedly high. Indeed, in Western countries, between 30 and 40% of women receive antibiotics either prenatally or during birth, with the majority prescribed for prophylactic reasons (111–113). This has real consequences on the neonatal microbiota. Infants with mothers exposed to antibiotics during pregnancy are reported to have reduced microbial diversity, less abundant Bacteriodetes and Bifidobacteria, and expanded γ-Proteobacteria (114, 115). Importantly, while the risks associated with antibiotic disruptions can be mitigated with breastfeeding, this may be less available to mothers of lower SES, particularly in countries with reduced access to paid maternity leave (52, 116).

Prenatal distress

The strong link between lower SES and prenatal distress is associated with several adverse health outcomes in offspring, such as preterm birth, low birth weight, and negative neurodevelopmental outcomes (117–119). Various changes have been reported between infant microbiota and different indicators of prenatal stress (e.g., subjective distress, cortisol level, precarity, prenatal anxiety, depression, and perceived stress), the most consistent being enrichment of γ-Proteobacteria and reduction in *Bifidobacterium*. Although reductions in species diversity have been reported, they have not been as consistent and appear to vary depending on the measure and timing of distress, as well as the age of the offspring (120, 121). These effects may be partially explained by altering gut microbial composition during pregnancy and transmission of disrupted vaginal microbiome at birth based on mouse models (122, 123).

Maternal smoke exposure

Low SES is a risk factor for smoking, and maternal smoking during pregnancy is associated with increased incidence of premature birth, childhood obesity, developmental delays, respiratory disease, and long-term morbidity among offspring (124–127). Evidence connecting maternal smoke exposure to the infant gut microbiota is limited, but consistent. A review of three studies found that infants exposed to maternal smoke had an increased Firmicutes richness, which was associated with an elevated risk of childhood overweight and obesity (128). Similar findings were independently observed in a recent study using the cohort data from the Canadian Healthy Infant Longitudinal Development (CHILD) study, Canada, which revealed that maternal smoking during pregnancy increases the risk of being overweight at 1 and 3 years, and this was mediated by an increase in Firmicutes diversity (129). Interestingly, although smoking cessation during pregnancy does not reduce the risk of offspring being overweight, exclusive breastfeeding does.

Utilizing the gut microbiome to tackle disparities in health

Given the impact SES-linked factors have on the infant microbiota and long-term health, prioritizing healthy microbiota development during infancy may alleviate health inequities for future generations. Considerable attention has been given in other reviews to highlight specific avenues that reduce disruptions, reinforce health-promoting microbial interactions, and replenish important species when they are lost (10, 130–132). We will highlight methods that can be broadly integrated into public health efforts to inclusively bolster infant microbiota development throughout our society.

Reduce: promote antibiotic stewardship

An increasing number of antibiotic stewardship programs (ASPs) have been successfully implemented worldwide, leading to reduced antibiotic use, improved clinical and microbiological outcomes and economic benefits, indicating the effectiveness of this public health intervention (133–136). While the public focus on antibiotic stewardship has been aimed at limiting antimicrobial resistance, an unintended benefit may be protecting vital microbial species within the infant microbiota, which reduce instances of childhood NCDs. Supporting this, our own findings linked recent declines in antibiotic prescriptions within British Columbia, Canada to lower rates of pediatric asthma, with the infant microbiota demonstrated to be the

likely mediator of this relationship (60). As additional public health ASPs are launched, it will be interesting to observe if these trends are recapitulated across communities.

Reinforce: support breastfeeding and access to donor milk

Despite the clear benefits of breastfeeding and recommendations to initiate within the first hour of life and exclusively breastfeed for the first 6 months, SES-disadvantaged families often have lower breastfeeding initiation and duration rates (137). This can be attributed to barriers such as a lack of breastfeeding educational resources, an absence of breastfeeding experience within previous generations, and employment and financial limitations (132, 138, 139). Some of these can be addressed at the individual or community level, as evidenced by randomized controlled trials that have successfully increased breastfeeding motivation and success (140, 141). However, societal-level policy and fiscal support, such as generous, universally paid parental leave, are needed to decrease the chasm between low- and high-SES families (132, 142). Furthermore, when breastfeeding is just not an option, regardless of support, standard care that includes access to donor breast milk or prioritized research into formulas that mimic human milk is needed to support the infant microbiota (143, 144). All of these in parallel are necessary to improve long-lasting cross-generational health outcomes and reduce health disparity.

Replenish: restore and boost exposure to missing microbes

Many researchers now believe that our society's intergenerational loss of microbiota biodiversity has paralleled the rise in NCDs (145, 146). This indicates that reinforcements, such as breastfeeding, alone may be insufficient and will need to be complemented by reintroducing key species to infants. Despite the efficacy of full microbiota replacement through FMT for severe diseases such as *Clostridioides difficile* in older populations, it is unlikely to serve as a preventative measure in infants (147). Even randomized controlled trials that effectively swabbed C-section born neonates with maternal vaginal microbes faced significant opposition from the medical community (148, 149). More feasible are live biotherapeutic products (LBPs) that contain rigorously controlled and tested microbial species to replenish key missing microbes. One excellent example is *B. infantis*, a crucial HMO-utilizing microbe that has declined in North America (95, 150–152). To combat this, community-based stool testing during checkups could identify infants who would benefit from a *B. infantis* LBP, which has been shown to successfully colonize infants to reduce gut dysbiosis and shape immune development (151, 153–155). Beyond *B. infantis*, *Bifidobacterium* and *Lactobacillus* contain a number of secure and effective species that can colonize both breastmilk and neonate gut microbiota when administered to mothers during pregnancy or lactation (156, 157). However, studies supporting their utility for long-term colonization and health benefits are still lacking (158, 159). In addition to targeted microbial restoration, a broader "rewilding" of urban environments could provide widespread and lasting benefits to counter biodiversity loss. Indeed, the soil microbiota found in revegetated urban spaces mimics that of natural vegetation, supporting the feasibility of reintroducing wild microbes into urban environments. Naturalized greenspaces can boost microbial biodiversity in young children, with measurable impacts benefitting tolerant immune responses (160, 161). In addition, there is more work to be done in public health policy to mitigate SES-associated microbiota and health inequity, such as making healthcare more affordable, providing education to the general public (i.e., hygiene hypothesis), and increasing access to fresh foods. Collectively, public health initiatives may be able to equitably restore necessary early-life microbiota interactions at the community level, thereby reducing or preventing microbiota-associated SES disparities and ensuring equitable health outcomes.

Conclusion

Over the past several decades, growing evidence has demonstrated the impact of early-life and cross-generational factors on infant microbiota development. These factors include delivery mode, breastfeeding, maternal stress, diet, and urbanization, all of which are commonly associated with SES. Since microbiota composition is modifiable and associated with infant immune development and long-term health, it represents an important opportunity to mitigate the impact of SES inequity on health disparity. In this context, broadly integrated biomedical and policy interventions aimed at reducing, reinforcing, and replenishing the disrupted microbiota should be adopted and provided at equalizing access. In addition, further studies aimed at understanding SES-associated microbiota diversity, composition, and functions in large-scale, diverse, and longitudinal settings are needed to better understand the variations in gut microbiota across diverse geographies, ethnicities, lifestyles, and ages.

Author contributions

DD: Conceptualization, Visualization, Writing – original draft, Writing – review & editing. CP: Conceptualization, Supervision, Visualization, Writing – review & editing. ST: Conceptualization, Supervision, Writing – review & editing, Funding acquisition, Project administration, Resources.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. S.E.T. holds a Tier 1 Canada Research Chair in Pediatric Precision Health and the Aubrey J. Tingle Professor of Pediatric Immunology. For this research, support was provided by the Canadian Institutes of Health Research (CIHR; EC1-144621), the Allergy, Genes and Environment Network of Centres of Excellence (AllerGen NCE) (12CHILD) and Genome Canada/Genome BC (274CHI). D.L.Y.D. is supported by a Canadian Institute of Health Research Frederick Banting and Charles Best Canada Graduate Scholarship Doctoral Award (CIHR CGS-D) and the University of British Columbia Four Year Doctoral Fellowship (4YF).

Acknowledgments

The authors acknowledge the value of ongoing conversations with members of the Turvey lab and the CHILD study in refining the concepts developed in this review. The authors acknowledge the support of Courtney Hoskinson in finalizing the article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

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frontiers | Frontiers in Allergy

TYPE Mini Review
PUBLISHED 25 October 2024
DOI 10.3389/falgy.2024.1455438

OPEN ACCESS

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RECEIVED 26 June 2024
ACCEPTED 07 October 2024
PUBLISHED 25 October 2024

CITATION
Donald K and Finlay BB (2024) Experimental models of antibiotic exposure and atopic disease.
Front. Allergy 5:1455438.
doi: 10.3389/falgy.2024.1455438

Experimental models of antibiotic exposure and atopic disease

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In addition to numerous clinical studies, research using experimental models have contributed extensive evidence to the link between antibiotic exposure and atopic disease. A number of mouse models of allergy have been developed and used to uncover the specific effects of various microbiota members and perturbations on allergy development. Studies in mice that lack microbes entirely have also demonstrated the various components of the immune system that require microbial exposure. The importance of the early-life period and the mechanisms by which atopy "protective" species identified in human cohorts promote immune development have been elucidated in mice. Finally, non-animal models involving human-derived cells shed light on specific effects of bacteria on human epithelial and immune responses. When considered alongside clinical cohort studies, experimental model systems have provided crucial evidence for the link between the neonatal gut microbiota and allergic disease, immensely supporting the stewardship of antibiotic administration in infants. The following review aims to describe the range of experimental models used for studying factors that affect the relationship between the gut microbiota and allergic disease and summarize key findings that have come from research in animal and *in vitro* models.

KEYWORDS

allergies, atopy & microbiome, gut microbiota, antibiotics – immune effect, animal models, cell culture models

Introduction

Experimental models enable the characterization of complex host responses ranging from cellular to systemic, contributing vitally to major advancements in immunology. Mice have long been the model of choice when studying host immunity and infection responses. The murine genome overlaps substantially with that of humans, and many key immune pathways are conserved. However, human and mouse immune systems are not identical, and findings in mouse models do not always translate directly to human application (1, 2). Non-animal models using cultured intestinal epithelial and immune cells, or miniature organ-like structures, have also been developed to determine specific effects of bacteria on various cell types (3, 4). Although this enables the study of cells derived specifically from humans, the response of cells grown in culture does not always reflect what occurs in the complex environment of a mammalian host. However, when used in conjunction with clinical human studies, both animal and *in vitro* models are critical to defining the mechanisms of host-microbiota interactions that affect immune development and atopic disease.

Mouse models for studying the gut microbiota

Mouse models enable the manipulation of a microbial community growing within the complex environment of a mammalian host. Standard laboratory mice are considered "specific-pathogen free" or SPF, because they have tested negative for a set of disease-causing pathogens. These mice are raised in clean laboratory conditions and have a microbiota that is lower in diversity than that of humans, but complex enough to simulate a stable gut community with some colonization resistance capacity (5). Microbiota composition differs depending on the mouse vendor and housing conditions, which can lead to differences in baseline immunity and response to treatments (6). Additionally, it can be difficult to tease apart direct effects of microbes on the host from indirect effects that act through the existing microbiota when studying SPF mice. Pretreatment with antibiotics is often required to promote stable colonization with a newly introduced microbe, which can confound results. Despite these caveats, SPF mice are used extensively to demonstrate the effects of antibiotics and microbiota alterations on immune development and disease (7–11).

As the microbiome field has grown, a variety of animal models have been developed to combat the issues faced with SPF mice (Figure 1). Germ-free (GF) mice, which are completely sterile, do not have a microbiota and can be more easily colonized than SPF mice (12). Mono-colonization of germ-free mice is commonly used to study the effects of one bacterial species at a time, independent of any confounding effects of other microbes in the environment. GF mice can also be colonized with a defined consortia of microbes to study simplified, specific communities and microbe-microbe interactions within the gut environment (5, 13, 14). Humanized mice, which are created by transferring human feces to GF mice, display a microbiota that is more diverse and complex than that of SPF mice and more similar to that of humans, facilitating the study of clinically relevant human-colonizing microbes *in vivo* (15, 16). However, some microbes found in humans require a host-specific niche and fail to colonize mice, limiting the utility of humanized mice (17). Lastly, transient colonization of mice with microbes that decline and disappear demonstrate the lasting effects of microbial exposure at specific developmental stages (18).

Mouse models of allergic disease

Several mouse models have been developed to study the atopic immune response, with allergic asthma and food allergy being most common (Figure 1). Since all atopic diseases involve the same key pathways (Th2 cell activation, IgE production, mast cell degranulation, basophil hyperplasia), the basic process of allergy induction is similar between models: mice are first sensitized and then challenged with an allergen to initiate and then stimulate a Th2-mediated response (19–21).

There are two popular models of allergic asthma. The OVA model involves intraperitoneal injection, followed by intratracheal or intranasal administration, of ovalbumin derived from chicken egg (22). The house dust mite (HDM) model involves a series of intranasal exposures to protein from a common HDM species such as *Dermatophagoides pteronyssinus* (23). Both models stimulate a Th2-mediated response and lung histopathology (22, 24).

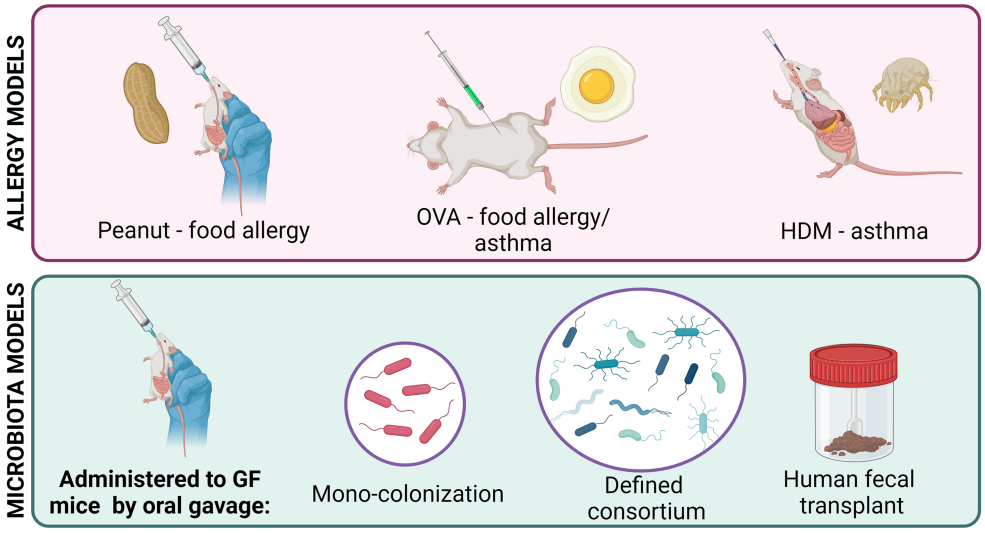

FIGURE 1
Common murine models for studying allergic disease and the gut microbiota.

Food allergy models are difficult to develop because oral exposure to even concentrated allergenic food proteins results in oral tolerance and fails to induce a response (25). For oral induction, food allergens must be administered with adjuvants to induce a Th2-mediated response (26). For example, concentrated peanut extract can be administered orally with cholera toxin, resulting in elevated IgE production and anaphylaxis (27). Peanut allergens can also be administered epicutaneously to mice to induce Th2-mediated responses which can be further stimulated orally in the absence of an adjuvant, which may be explained by the link between skin barrier function and food allergy (28–30). Although peanut proteins show the highest allergenicity in mice, egg and milk allergies have been induced using adjuvants as well (26).

There are also models of atopic dermatitis and allergic rhinitis, the common forms of which also involve sensitization and challenge with ovalbumin, but are less common (31, 32). In humans, atopic dermatitis often precedes the development of food allergy and asthma in a sequential pattern termed the "atopic march" (33). The atopic march has been demonstrated in the NC/Nga mouse, commonly used to study atopic dermatitis (34). Epicutaneous OVA or peanut protein sensitization can also induce allergic manifestations at other sites (35). Our understanding of the microbiota's role in the atopic march is lacking, and murine models that display multiple allergies initiated via the skin should be utilized to further investigate factors that affect the progression of disease.

Studies of the microbiota and antibiotics using allergy models will be highlighted below.

Atopic disease—insights from germ-free mice

GF mice, which lack a microbiota entirely, display a dramatically altered immune system (12). Intestinal and systemic immune compartments are largely underdeveloped or missing in GF mice. While some of these phenotypes can be rescued by colonization of germ-free mice at any age, others require colonization within the critical early-life window.

As in newborns, the immune systems of GF mice are skewed toward Th2 responses. Th1 and Th2 cells reciprocally regulate each other, and in the absence of microbial stimulation of Th1 pathways, Th2 responses go uncontrolled (36). This phenomenon supports the hygiene hypothesis, which posits that increased Th2 responses can be attributed to reduced microbial exposure and Th1 stimulation in modern, more hygienic environments (37, 38). Although mono-colonization of germ-free mice with certain commensals can reduce Th2 skewing, full restoration of normal T cell proportions requires colonization with a diverse microbiota (39).

Germ-free mice also display increased invariant natural killer T (iNKT) cells in the lung and colon. iNKT cells are found in higher proportions in individuals with severe and uncontrolled asthma (40). Concordantly, germ-free mice display increased iNKT-mediated inflammation in response to asthma models (41). Exposure to a specific antigen produced by the common commensal *Bacteroides fragilis* within the first two weeks of life is sufficient to rescue iNKT cell levels (42). iNKT cell levels cannot be restored in adult mice, implicating the preweaning period as an important moment in immune imprinting by the colonizing microbiota (12).

Serum IgE titers are also elevated in germ-free mice (43). This contributes to more severe anaphylaxis in food allergy models, and can only be rescued by colonization in the first few weeks of life (12, 43). Regulatory T cell development also requires antigenic exposure specifically during the early life period (44, 45). Many studies have demonstrated that these immunological features make GF mice more susceptible to asthma models (46–48). However, others have shown that certain aspects of the GF mouse allergy response are dampened (49), and that colonization of certain species can worsen allergy symptoms of germ-free mice (50). Together, studies in germ-free mice have shed light on the numerous pathways by which microbes in the gut can protect against or contribute to asthma and allergy development.

The weaning reaction in mice

While colonization of GF mice at any age can rescue some of their immune alterations, exposure to microbes during the early-life window is required for complete restoration of proper immune function. Recently, this window has been well-defined in mice to be the first 3 weeks of life. At weaning, mice undergo a dramatic immune reaction to the influx of microbes and loss of passive immune factors in milk, which imprints their immune systems and susceptibility to disease for life (51). Although it is not clear whether the "weaning reaction" occurs in humans, the associations of breastfeeding and microbiota maturation in infancy with immune health later in life suggest that the human weaning period is also critical for microbiota and immune development (52, 53).

At weaning, the mouse microbiota diversifies and expands, displaying a bloom in Clostridia and Bacteroides which replace gamma-Proteobacteria and Lactobacillus species. This is similar to the microbiota shifts observed in humans from birth until 6 months of age (21, 54, 55). Milk contains several anti-inflammatory molecules, which gradually decline in concentration over time post-gestation. Epidermal growth factor (EGF) in milk delays the opening of Goblet-cell-associated antigen passages (GAPs) in the gut. As EGF levels decrease over the course of the first few weeks of life, GAPs open and allow for microbes to interact with and stimulate the immune system. In response to the developing microbiota and mucosa, GAPs close shortly after weaning. Antigenic exposure through GAPs drives regulatory T cell development, promoting tolerance of the gut microbiota and imprinting the immune system. This process is unique to the early-life period and cannot be induced in adult mice. Importantly, the weaning reaction protects against allergic inflammation later in life, and antibiotic exposure before but not after weaning significantly disrupts immune programming and contributes to worsened health outcomes (51).

The weaning reaction has not been demonstrated in humans, and while the exact mechanism may not be the same, the

weaning reaction theory provides a potential explanation for the relationship between neonatal microbiota disruption and adverse health outcomes that is well-observed in humans. Accessing human neonatal tissue and blood to define a human weaning reaction would be immensely difficult. However, the deep characterization of this response in mice along with ample evidence of long-term effects of early-life antibiotics in infants provide clues to the process of microbe-mediated immune imprinting that occurs in humans.

Antibiotics, the microbiota, and mouse models of asthma

Prompted by associations observed in human cohorts, the relationship between the gut microbiota and asthma has been studied and characterized extensively through colonization of GF mice or supplementation of SPF mice with different combinations of bacteria.

Numerous animal studies have identified specific immunomodulatory bacteria that protect against allergies. As mentioned above, colonization of germ-free mice with allergic infants is sufficient to increase anaphylaxis susceptibility (56). Through sequencing and host gene expression analysis, this effect was explained by protective effects of the Lachnospiraceae species, *Anaerostipes caccae*. Mono-colonization experiments confirmed that this species alone, which is elevated in healthy infants, contributes to oral tolerance and protects against anaphylactic responses. In another study, four genera of bacteria inversely associated with allergies in human infants were administered to mice and shown to ameliorate OVA-induced asthma (57). This was linked to the effects of specific bacterial metabolites on immune development.

Species of Bifidobacteria and Lactobacillus, which are known to be promoted by breastmilk and are inversely associated with allergies, have also been tested in animal models (58–60). Supplementation with *Bifidobacterium longum* and *Bifidobacterium breve* have been shown numerous times to limit allergic responses to various models by inducing regulatory T cells and dampening Th2 responses (48, 61–63). Lactobacillus species have also been shown to improve intestinal barrier integrity and promote Th1 responses, which limit allergic phenotypes (64, 65).

In all of these studies, bacteria known to be associated with health in humans were investigated and causally linked to allergy protection. Animal models have been key to moving from correlation to causation in our understanding of the multiple roles of the microbiota in immune development and have helped identify the specific bacteria and pathways that should not be disrupted during the neonatal period. In addition to providing insight into protective and beneficial bacteria, animal studies have demonstrated the effects of early-life antibiotic exposure on asthma.

Vancomycin treatment during neonatal but not adult life increases susceptibility to OVA-induced asthma in mice (8). This is linked to alterations in gut microbiota composition and diversity, and reduced colonic Treg cell levels. Both of these phenotypes were more drastic in neonatal than adult vancomycin-treated mice, and the period between birth and weaning was identified as the window during which antibiotic induced dysbiosis was found to significantly affect adult asthma outcomes (66). Similarly, Azithromycin treatment in early life increased IgE and Th2 responses to HDM-induced asthma (67). Interestingly, in this study, transfer of the azithromycin perturbed microbiota to adult germ-free mice did not transfer the phenotype. However, the offspring of these mice displayed worsened asthma outcomes, indicating that the effects of microbiota alterations on immune development must occur in early life. Dysbiosis induced by a combination of antibiotics has also been shown to impair oral tolerance by disrupting dendritic cell development in the gut (68).

Exposure to a single course of macrolide antibiotics at a clinically relevant dose during neonatal life is sufficient to reduce microbial diversity and shift community composition, which persists into adulthood. This was accompanied by permanent dampening effects on local and systemic immunity (69). In contrast, adult mice treated with the same antibiotic course displayed rapid microbiota recovery and did not show immune aberrations. Microbiota transfer from antibiotic-treated mice to germ-free mice conferred the altered immune phenotype in this study, demonstrating that the perturbed microbiota is sufficient to drive immune alterations associated with antibiotic exposure. Lynn et al. treated mice with ampicillin and neomycin until weaning and then aged them to 700 days (70). They found that early-life antibiotic exposure affects immune status, longevity, and metabolism even long after antibiotic exposure.

The studies highlighted above demonstrate the multitude of detrimental effects that antibiotic exposure in early life have on long-term health. Mouse studies have been vital in our understanding of both the specific effects of "protective" microbes and antibiotics on different facets of immune development, and the uniqueness of the infancy period in these processes. They provide strong support for limiting antibiotic administration in infants whenever possible.

Non-animal models for studying antibiotics and allergic asthma

In addition to animal models, cultured human cells are frequently used to investigate the microbiota and allergic responses. Epithelial responses to microbial products can by studied by monoculture of human intestinal or lung epithelial cells. For example, Bifidobacteria species grown on human milk oligosaccharides induce anti-inflammatory responses in human-derived intestinal cells (71). Some commensal species and their metabolites have also affect barrier integrity of intestinal cell monolayers (72–75), either promoting or impairing barrier function, which has implications for allergy and asthma susceptibility (76, 77). However, allergic disease involves communication between the epithelium, the mucosal immune system, and systemic circulation, and it is difficult to define the mechanisms and effects of microbe-immune crosstalk using only

one cell type. Co-culture systems involving epithelial cells grown with dendritic cells, macrophages, and lymphocytes have been developed to combat this issue.

In one study, peripheral blood mononuclear cells (PBMCs) were isolated from atopic or non-atopic individuals and co-cultured with epithelial cells exposed to microbial antigenic stimulation to demonstrate that commensal microbe exposure limits the allergic response (78). Co-culture of PBMCs with intestinal epithelial cells has also been used to mechanistically link Bifidobacteria and other commensal species to regulatory T cell responses (79, 80). In another study, human-derived naïve T cells were exposed to fecal water from infants that received *Bifidobacterium infantis* supplementation or fecal water from control infants. This enabled the identification of a specific microbial metabolite produced by B. *infantis* in the infant gut that skews T cell phenotypes away from allergic (Th2 and Th17) and towards Th1 phenotypes (81). More recently, co-culture systems involving many cell types have been developed. Zuurveld et al. designed a model involving epithelial cells, dendritic cells, T and B cells, and mast cells and were able to simulate the entire allergic pathway from epithelial cell allergen exposure to mast cell degranulation and IgE production *in vitro* (4).

Finally, in addition to mono- and co-culture systems, 3D structures that better simulate organ-level biology can model microbe-immune communication. Organoids and gut-on-a-chip devices are derived from human intestinal stem cells and morphologically mimic 3D intestinal tissue, displaying crypts, villi, Paneth cells, mucous production, and distinct apical and basolateral compartments (3, 82). These models are only beginning to be used to study microbe-host interactions that influence systemic health but offer a promising physiologically relevant alternative to animal models (83, 84).

Conclusions

Experimental models have characterized the complex and dynamic processes of microbe-mediated immune development that occurs in early-life and validated key taxa that drive protective and beneficial immune processes. They have provided ample evidence that antibiotics have detrimental effects on gut microbiota composition, long-term health, and allergy in a controlled setting. They have also defined the features of immunologic development that occur during the critical early-life window, and illustrated the imprinting effects of the microbiota during this period. While lab rodents and cultured cells do not replicate the human gut and immune system identically, the findings highlighted above strengthen and complement conclusions from human association studies, drawing clear mechanistic links between antibiotic exposure, reduced microbiota diversity, and allergic outcomes.

Author contributions

KD: Writing – original draft, Writing – review & editing. BF: Writing – original draft, Funding acquisition, Supervision.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. KD is supported by the Four Year Fellowship Tuition Award, President's Academic Excellence Initiative PhD Award and International Tuition Award at the University of British Columbia.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Public Health

TYPE Mini Review
PUBLISHED 06 November 2024
DOI 10.3389/fpubh.2024.1471252

OPEN ACCESS

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RECEIVED 26 July 2024
ACCEPTED 16 September 2024
PUBLISHED 06 November 2024

CITATION
Loutet MG (2024) Individual- and system-level determinants of breastfeeding in a low-resource setting.
Front. Public Health 12:1471252.
doi: 10.3389/fpubh.2024.1471252

Individual- and system-level determinants of breastfeeding in a low-resource setting

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The benefits of breastfeeding are widely established and therefore the World Health Organization recommends that every child be exclusively breastfed for the first 6 months of life and continue breastfeeding up to 2 years of age or beyond. However, the rate of exclusive breastfeeding is low globally and has declined in Bangladesh in recent years. In this review, Bangladesh is used as an example to demonstrate the complex individual- and system-level determinants of breastfeeding in a low-resource setting. Mothers face barriers to breastfeeding within the context of marketing by commercial milk formula companies, limited safe alternatives to breastfeeding directly from the breast, and insufficient resources to support breastfeeding in the hospital, community, and workplace setting. Future research and implementation science is required to investigate the overlapping effects between breastfeeding and the high antibiotic use and Caesarean section rates in Bangladesh, along with public health efforts to promote breastfeeding based on robust evidence.

KEYWORDS

breastfeeding, Bangladesh, low- and middle-income country, public health, individual-level determinants, system-level determinants

Introduction

The World Health Organization (WHO) recommends every child be exclusively breastfed for the first 6 months and continue to breastfeed up to two years of age or beyond, while introducing complementary foods starting at 6 months. However, rates of exclusive breastfeeding to 6 months of age remain low globally, with only 48% of infants ever exclusively breastfed below 6 months of age in 2022 (1). In Bangladesh, 53% of infants below 6 months of age were exclusively breastfed based on the 2022 Demographic and Health Survey (DHS) and the rate of exclusive breastfeeding has remained relatively stable between 53 and 65% over the past decade (2, 3). However, rates of infants exclusively breastfed up to 6 months of age are likely even lower than these estimates because the DHS collects data on infant feeding practices using a single (cross-sectional) 24-hour recall from caregivers of infants below 6 months of age, which does not capture the dynamic nature of early-infant feeding with periods of time when an infant may be breastfeeding, formula-feeding, or being given other liquids that is common in low- and middle-income countries (LMICs) (4). An observational study conducted in 8 LMICs showed that using the WHO indicator (which uses DHS data) the proportion of infants 0–5. 9 months who exclusively breastfed was 71% among Bangladeshi infants compared to only 10% of infants were exclusively breastfed up to 6 months of age (i.e., met the WHO recommendation) using longitudinally collected 24-hour recalls of infant feeding practices biweekly from shortly after birth to 6 months of age (4).

It is widely established that breast milk contains multiple components that support infant's growth and development of their immune system and gut microbiome (5–7). Exclusive breastfeeding in particular is important in LMICs because exclusive breastfeeding prevents

infections through consumption of contaminated non-human milk feeds and suboptimal breastfeeding causes an estimated 600,000 annual child deaths from pneumonia and diarrhea alone (8, 9).

Recent evidence suggests that breastfeeding may mitigate the effect of antibiotics on the infant gut microbiome, and thereby confer a protective effect against antibiotic-associated risk of asthma (10, 11). This is especially important in a context such as Bangladesh where antibiotic-use and the prevalence of atopic dermatitis and asthma among infants are high (12–15). In a study of longitudinal birth cohorts from 8 LMICs, the average antimicrobial courses per child-year up to 2 years of age was double in the Dhaka, Bangladesh birth cohort compared to the global cohort, 10.3 and 4.9, respectively (13). By 6 months of age, over 98% of infants in the Dhaka birth cohort had received antibiotics. Furthermore, a study of inpatient antimicrobial prescribing among infants 0–12 months in Dhaka found that antimicrobials were prescribed in 73% of admissions (14). The study also assessed if the use of antimicrobials was appropriate based on the use of 'access' (should be widely available, affordable and quality assured), 'watch' (high resistance potential and should be limited) and 'reserve' (last resort in highly specific patients to preserve effectiveness) antimicrobials. Overall, 58% of antibiotics that were prescribed were classified as 'access', 38% as 'watch' and 1% as 'reserve; with 'watch' antimicrobials used in 26% of neonatal sepsis cases and 76% of lower respiratory tract infection admissions. Antimicrobials were also used in 51% of gastroenteritis and 28% of neonatal jaundice admissions, which were likely viral illnesses or conditions for which antibiotics do not usually confer benefit. Finally, among infants aged 2–6 months brought to a hospital in Dhaka for management of diarrhoeal illnesses, 52% had received antibiotics before hospital admission to treat the diarrhoeal disease for which they were seeking medical attention, possibly due to the fact that medicines are available from diversified sources and antimicrobials may be obtained without physician prescription (15). However, this phenomenon is not unique to Bangladesh, as a study of over 3,000 hospitalized infants less than 60 days of age from 11 countries (mainly Asia and Africa), showed that 98% received antibiotics, with the majority considered 'watch' (66%) (16).

This highlights the need for research efforts to focus on promotion and support for breastfeeding based on robust evidence of associations with modifiable risk factors. Although there are known biological determinants of breastfeeding such as gestational age, birthweight, and other maternal and infant health conditions, this paper will focus on structural—both at the individual- and system-level—determinants of breastfeeding in Bangladesh and the main settings that are influenced by these determinants: health systems, communities, workplaces, governments, and commercial. These determinants and settings are the focus of this paper due to their modifiable—although complex—nature and potential for intervention. These challenges are present in many LMICs (17), but Bangladesh is presented in this paper as an example.

Hospital-level determinants

Bangladesh has the highest rate of live births by Caesarean section (C-section) within health institutions among countries where less than 60% of births were institutional births (18) and in 2022, 45% of live births in the 2 years preceding the DHS-2022 were delivered via C-section (3). C-sections have been shown to be associated with lower rates of mother-infant skin-to-skin contact immediately after birth (19–22), which further significantly reduces rates of breastfeeding initiation (23, 24) and exclusive breastfeeding at 3 and 6 months (25). Although C-sections provide an important life-saving intervention to mothers and newborns, they also result in an increased risk of complications, with 62% of mothers experiencing severe acute morbidity resulting from a C-section in LMICs compared to less than 1% in HICs, and long-term consequences for mother and child (26–30). For instance, being born vaginally has been associated with a lower risk of atopic disease in infants compared to those born by C-section (31). Therefore, C-sections should only be used when medically indicated (32).

Rates of C-section use have increased greatly in LMICs, which may reflect an increase in the number of women giving birth in medical institutions, an increase in access to C-sections, but also possibly a change in the distribution of reasons for C-sections. There is a dearth of evidence on the reasons for C-sections in Bangladesh, but risk factor analyses using the Bangladesh DHS from 2017 to 2018 reported that C-section delivery was higher among women with higher education, from wealthier households, urban areas, and those with access to media (33, 34). Looking to other LMICs, a study among nulliparous women in Argentina found that mode of delivery preferences were most strongly influenced by a doctor or midwife, and that sociodemographic factors such as socioeconomic status and age played a strong role in determining the extent of their influence (35).

Pregnant people and new mothers in Bangladesh receive breastfeeding support while attending health facilities during antenatal care and within 2 days of giving birth; however, access is far from universal (36–38). In 2001, WHO and UNICEF launched the Baby-Friendly Hospital Initiative (BFHI) so that hospitals could be accredited for their commitment to protect, promote, and support breastfeeding (39). An influential cluster-randomized trial called Probit, conducted in Belarus from 1996 to 1998, modelled the intervention on the BFHI and found a large significant effect on any breastfeeding at 12 months (20% vs. 11% in controls) and exclusive breastfeeding at 3-months (43 vs. 6% in controls) and 6-months (8% vs. 0.6% in controls) (40). Even though Bangladesh has made strides in implementing BFHI, the latest report in 2016 showed only 1.5% of hospitals had the designation and reported challenges with training and funding (41, 42). In 2012, the Bangladesh Ministry of Health and Family Welfare established a partnership with the Bangladesh Breastfeeding Foundation (BBF), a non-governmental organization (NGO), to provide technical support to the BFHI programme through capacity building, training, and monitoring (43, 44).

A promising approach to improve breastfeeding support in facilities is increasing the midwife workforce, which is a critical part of Bangladesh's sexual, reproductive, maternal, newborn, and adolescent health (SRMNAH) strategy (45). In 2008, Bangladesh upgraded its midwifery workforce to meet global standards, with 8,000 registered midwives deployed to government hospitals, NGOs in humanitarian settings, and in private facilities (46). A 2019 qualitative evaluation of interviews with maternity ward staff from government sub-district hospitals in Bangladesh where midwives had been deployed compared to hospitals without midwives indicated that midwives improved quality of care through interventions including skin-to-skin contact, breastfeeding, and obstetric emergency and post-partum management (47). By 2023, midwives in public hospitals were

in charge of labour wards and attended up to 85% of births, which led to significant increases in positive birth practices, including use of antenatal card and partograph, upright birth positioning, and mother-infant skin-to-skin contact (46, 48).

Community-level determinants

Bangladeshi mothers receive support for breastfeeding within the community from a variety of formal (i.e., through funded programmes) and informal (i.e, family and other community members) sources. Formal programmes have been informed by evidence; however, sustainability, standardization and scale-up remain a challenge nationally (49, 50). For instance, a 2-year follow-up to Alive & Thrive's intensive community-based infant and young child feeding (IYCF) intervention implemented by the local NGO BRAC in rural Bangladesh showed that exposure to some aspects of the intervention had decreased significantly after external funding support from the initial donor agency ended (51). Although there was a sustained impact on early initiation and exclusive breastfeeding along with other IYCF practices and knowledge in areas that received the intervention compared to those that did not, there had been no major scale-up of the intervention to areas that did not receive the initial intervention. Another example is the BBF-led program to implement Mother Support Groups to improve maternal and child nutrition at the community level (52), which is supported with evidence from studies using peer support for lactating women in Bangladesh to effectively improve exclusive breastfeeding to 6 months of age in both urban (53) and rural (54) settings, and specifically among factory workers (55).

Similar to hospital-based breastfeeding support, the increase in the midwife workforce in Bangladesh poses an opportunity for community-level support because midwives can work in the community providing information and breastfeeding support. This is currently being done by midwife students, but overall qualitative evidence from midwives shows that the midwifery centre care model is inaccessible to communities due to challenges of traditional practices and the need for wider acceptance of the midwifery-led care model (56). A study using the Lives Saved Tool modelled the effect of scaling-up the coverage of health interventions delivered by professional midwives on a number of maternal and health outcomes including mortality and exclusive breastfeeding and found that increasing the target coverage of breastfeeding promotion as part of midwives scope of work would have a significant effect on the proportion of children aged 1–5 months who are exclusively breastfed (an increase from 37 to 55% in the lowest human development index (HDI) countries) (57). To note, Bangladesh was categorized as a low-to-medium HDI country and data on exclusive breastfeeding was not presented in the paper on all country groups, but substantial scale-up of midwife interventions had the greatest impact on maternal and neonatal death in the low-to-medium HDI countries, which account for a large proportion of the worlds populations and have high baseline mortality rates.

In Bangladesh, social factors within the community such as family structure and gender norms also impact the breastfeeding journey. A systematic review of facilitators and barriers to early initiation of breastfeeding in South Asia reported that influence of a mother-in-law on maternal and newborn care and lack of the mother's involvement in decision-making were barriers to early initiation of breastfeeding in Bangladesh (58). Qualitative evidence from interviews with Bangladeshi mothers also identified pressure from older adult family members to feed their infants commercial milk formula (CMF), water and semolina and to prioritise other household chores as barriers to exclusive breastfeeding (59, 60). There is some qualitative evidence that mothers in Bangladesh consider the sex of the infant when deciding to continue to breastfeed due to differences in infant behaviour (59); however, this is not supported by large quantitative national surveys that show no difference in breastfeeding practices by infant sex (3, 61, 62). Importantly, in a study of parents with a child 0–6 months of age from a nationally-representative sample in Bangladesh, fathers' knowledge about exclusive breastfeeding and support to mothers to practice exclusive breastfeeding had a significant positive impact on maternal exclusive breastfeeding knowledge and attitude (63). Consideration of these cultural and social factors is essential to designing breastfeeding promotion interventions because for instance, male engagement does not consistently improve rates of exclusive breastfeeding across all LMICs with different gender norms (64). In Bangladesh, Alive & Thrive's intensive IYCF intervention that successfully increased early initiation and exclusive breastfeeding included mass media campaigns targeted at mothers, fathers, and community leaders (65, 66).

Female labour force participation

Returning to work is one of the top reasons for not breastfeeding reported by new mothers in LMICs (67). In LMICs, where a high proportion of work is in the informal sector, underpaid and unprotected, many women face the challenge of competing priorities between the time required to breastfeed and other care and income-earning responsibilities (68). Workplace support for breastfeeding includes low-cost strategies that are cost-effective, especially given the high value of breastfeeding such that in 2020 the global monetary value of women's milk production among infants 0–36 months was approximately $US 3.6 trillion (68–70). To create an enabling workplace environment for breastfeeding, a study in South Africa developed a comprehensive practice model based on critical review of the literature, mixed-methods data collection and consensus from experts, which was centered on time, space and support inputs by the employer, measurable outputs, and short to long-term outcomes (71). The methodology, and possibly even the practice model itself, has the potential to be applicable in other LMIC settings.

In Bangladesh, female labour force participation has been increasing (26% in 2003 to 36% in 2016) (72) and 85% of the estimated 4 million garment factory workers are women of reproductive age (73). However, these women lack information and support to continue breastfeeding when they return to work. In a pooled analysis of the Bangladesh DHS from 2011 to 2018, employed mothers had 24% lower odds of any exclusive breastfeeding between 0–5.9 months (adjusted odds ratio = 0.76, 95%CI: 0.59–0.96) (74). A survey in two factories in Dhaka, Bangladesh found that only 17% of female factory workers with infants aged below 2 years exclusively breastfed their infants up to 6 months of age (75). Furthermore, qualitative research among garment factory workers who were mothers of 0–12 month old infants showed that mothers introduce CMF as early as 2 months postpartum because they had very little knowledge about the use of expressed breast milk, did not have access to breast pumps or refrigeration at work or home, and were concerned about pathogenic contamination of expressed human milk due to this lack of refrigeration (76). Additionally, these

mothers faced barriers to breastfeeding due to excessive workload without scheduled breaks, inadequate child-care facilities at work, and caregivers at home who, understandably, were unable to bring the infants to the factories for feeding.

There have been a number of promising interventions to support Bangladeshi mothers returning to work. In a two-group longitudinal mixed-methods study comparing the effectiveness of a home-based peer support programme from 6 months of pregnancy until 6 months postpartum among pregnant and lactating factory workers compared to their unemployed female neighbors in Bangladesh, exclusive breastfeeding at 6 months was high among both groups (86% in employed group and 95% in unemployed group) (54). Peer counsellors in that study educated mothers on safe expression, storage and feeding of breast milk, which enabled employed mothers to feed infants their breast milk exclusively when returning to work and encouraged family members to trust and help throughout the process. However, employed mothers still described challenges with finding the time and space in the workplace to express their breastmilk. Recently, there has been more organizational commitment to support working mothers in Bangladesh. In 2016, UNICEF and the Bangladeshi government including the Ministry of Health and Family Welfare and Ministry of Labour and Employment convened a task force to improve maternity protection and infant and child care in Bangladeshi businesses (75, 77). UNICEF also supported the establishment of an advocacy programme to strengthen maternity rights and protect breastfeeding in the workplace called Mothers@Work. This initiative partnered with two factories in Bangladesh to act as pilot projects to support breastfeeding in the workplace (78). Most recently, the UNICEF-led Mothers@Work initiative partnered with Bangladeshi factory associations to support factories to provide breastfeeding spaces and breaks, childcare facilities, paid maternity leave, and a safe work environment for working mothers and pregnant women (79).

Commercial milk formula use

Overall, due to the high expense of CMF, breastfeeding is more prevalent in LMICs than high-income countries (5), and within LMICs, CMF-use is positively associated with household wealth (80). In Bangladesh, rates of CMF use are highest among the wealthiest households (17.4% from DHS-2014), but rates are still high among the poorest (9.5% from DHS-2014) (81) for which the risk and consequences from unsterile bottles and/or contaminated water supply is greatest (5, 9). The rate of CMF use is also increasing in Bangladesh, with 22% of infants aged between 0 and 5 months received mixed milk feeding (breast milk and CMF and/or fresh, packaged, or powdered animal milk) based on the DHS-2022, which was highest among the wealthiest households (24%) and lower among households in the lowest wealth quintile (13%) (3). Furthermore, a recent report by WHO and UNICEF from 2022 showed that 27% of women surveyed in Bangladesh were exposed to CMF marketing and 57% received recommendations from health professionals to use CMF products (82).

In 1981, the WHO developed the International Code for the Marketing of Breast-milk Substitutes and called on countries to enact individual laws and regulations to limit the marketing methods for CMF and related products (83). However, as of 2023, only 32 countries have legal measures substantially aligned to the Code and there has been an increase in advertising expenditure by CMF manufacturers by 164% in the past decade (84, 85). Violations of the WHO International Code are a global challenge and there is a substantial gap in multilevel and multicomponent interventions to address them (17, 68, 84–86). LMICs that have made progress against CMF markets, such as the Philippines, have done so through political commitment including an official database of reported violations of the Code and coalitions to resist the CMF industry (17, 68, 85). Although the Bangladesh Breastmilk Substitutes Act was developed and adopted by the Bangladeshi Parliament in 2017, the rates of exposure to CMF marketing and use of CMF highlight the need for robust implementation, stronger enforcement, and monitoring of the Act to protect families at all socioeconomic levels from unsubstantiated claims about CMF (87).

Conclusion

Factors affecting breastfeeding practices are often structural, socioeconomic, cultural, biological, and medical; and therefore, breastfeeding promotion requires multifaceted public health efforts to target these factors (17, 68, 69). Breastfeeding rates are impacted by all of these factors in Bangladesh within the context of marketing by CMF companies, limited safe alternatives to breastfeeding directly from the breast, and insufficient resources to support breastfeeding in the hospital, community, and workplace setting. The WHO cautions against the use of feeding bottles and breast-milk substitutes, especially in LMICs, due to the high risk of introducing contamination that can lead to life-threatening infections in young infants (88), so the fears expressed by Bangladeshi mothers of returning to work and risk among low-income households (81) are warranted. Encouragingly, the rate of exclusive breastfeeding is higher in Bangladesh compared to other countries worldwide. The overlapping effects of high antibiotic use and C-section rates may have even larger negative effects on infant health outcomes such as atopic disease if it were not for the protective effect of breastfeeding in this context; however, more research on these interrelated factors is required.

Author contributions

ML: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. The Author is a PhD candidate and receives support for her doctoral studies from the Canadian Institutes of Health Research Frederick Banting and Charles Best Canada Graduate Scholarship Doctoral Award.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Allergy

TYPE Original Research
PUBLISHED 23 December 2024
DOI 10.3389/falgy.2024.1491985

OPEN ACCESS

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RECEIVED 05 September 2024
ACCEPTED 03 December 2024
PUBLISHED 23 December 2024

CITATION
Lee TY, Petkau J, Saatchi A, Marra F, Turvey SE, Lishman H, Patrick DM, Cragg JJ, Johnson KM and Sadatsafavi M (2024) Impact analysis of infant antibiotic exposure on the burden of asthma: a simulation modeling study.
Front. Allergy 5:1491985.
doi: 10.3389/falgy.2024.1491985

Impact analysis of infant antibiotic exposure on the burden of asthma: a simulation modeling study

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Background: Infant antibiotic use is associated with increased risk of asthma. We examined the population impact of antibiotic exposure in the first year of life on the burden of pediatric asthma in British Columbia, Canada, using simulation modeling.
Methods: We performed a Bayesian meta-analysis of empirical studies to construct dose-response equations between antibiotic exposure in the first year of life and pediatric (<19 years of age) asthma. We used administrative health data to document trends in infant (<1 year of age) antibiotic use in British Columbia during 2001 and 2018 (the study period). An independently developed microsimulation model of asthma was utilized to estimate asthma-related outcomes under three scenarios pertaining to the trends in antibiotic use during the study period: (1) observed trends, (2) flat trend in which the prescription rate remained at the 2001 value, and (3) intermediate trends midway between these two. We reported cumulative person-years with asthma, cumulative asthma incidence, and cumulative asthma exacerbations among the pediatric population during the study period.
Results: There were 773,160 live births during the study period, with an average antibiotic prescription rate of 523 per 1,000 infants in the first year of life. The prescription rate decreased by 71.5% during the study period. In Scenario 1, there were 1,982,861 person-years with asthma, 183,392 asthma incident cases, and 383,072 exacerbations. Had the antibiotic exposure remained at the 2001 values (Scenario 2), there would have been additional 37,213 person-years with asthma, 10,053 asthma incident cases, and 23,280 exacerbations. Had the decline been half of the observed trend (Scenario 3), there would have been additional 20,318 person-years with asthma, 5,486 asthma incident cases, and 12,728 exacerbations. At least 80% of the excess burden in each outcome was attributable to the younger pediatric population of <10 years of age.
Conclusions: The decline in infant antibiotic exposure has resulted in a substantial reduction in the burden of asthma in British Columbia. Such benefits should be considered when evaluating the value proposition of initiatives aimed at reducing unnecessary antibiotic exposure in early life.

KEYWORDS

asthma, antibiotics, early life, projection, simulation modeling

1 Introduction

Overuse of antibiotics is a global health problem, with approximately 30% of outpatient antibiotic prescriptions estimated to be unnecessary (1). Many health jurisdictions across the world have undertaken initiatives, such as launching antimicrobial stewardship programs, to reduce unnecessary use of antibiotics. A recent systematic review of 52 studies reporting on such stewardship programs found an overall 10% attributable reduction in antibiotic prescriptions (2). However, it is not clear to what extent such a reduction will impact the health of the population. Understanding the population and policy implications of interventions is necessary for evidence-informed and efficient decision-making (3).

A prime example of the benefit of reduced antibiotic exposure is the potential reduction in the burden of asthma. Asthma is a common chronic inflammatory airway condition characterized by airflow restriction, heightened airway responsiveness, and structural alterations in the air passages (4). Asthma continues to present a public health challenge, affecting over 262 million people globally (5, 6). Numerous studies have shown asthma to result in premature mortality, diminished quality of life, and substantial economic burden (7).

Growing evidence shows a link between infant antibiotic use and childhood asthma development (8). This link can be attributed to the deleterious impact of antibiotics on the maturation of the infant gut microbiome, the composition of which plays a crucial role in healthy immunological development. Disruption of this process can lead to hyperinflammatory immune responses later in childhood and the development of allergic diseases such as asthma in later life (9–11). Therefore, it is plausible that the concomitant reduction in antibiotic use and the incidence of asthma observed in many jurisdictions may be related (11–14). For example, a 73% reduction (from 868 to 236 prescriptions per 1,000 population) in antibiotic use among infants was paralleled with a 41% reduction in asthma incidence (from 29 to 17 incident cases per 1,000 population) among children aged 1–4 years between 2000 and 2018 in British Columbia (15, 16). A concern about such associations is potential confounding by indication or reverse causation due to respiratory infection since antibiotics are indicated for respiratory infections, and respiratory infections are a risk factor for asthma (17). However, the association with asthma remains significant after adjusting for respiratory infections, and is also observed when restricting the outcome to non-respiratory indications (11). Moreover, at the ecological level, dramatic falls in childhood asthma rates following reductions in antibiotic use were not preceded by concomitant changes in the population prevalence of respiratory infections (11). The established pathway through missing taxa, altered metabolites and T cell development adds biologic plausibility for a causal association (18). Putting these together, preventing the early steps in asthma pathogenesis during infancy through reduction in antibiotic exposure presents a unique opportunity for asthma prevention (19).

Given the strength of evidence towards the causal association between antibiotic exposure and risk of childhood asthma, we sought to investigate the policy implications of reducing unnecessary antibiotic use. Focusing on British Columbia (BC), a Canadian province with a 2022 population of 5.4 million (20), our study aimed to address the question: "*What would the burden of asthma have been, had infant antibiotic exposure been different than observed?*" Specifically, the objective of our study was to project population-level asthma-related outcomes under different counter-factual scenarios related to recent declining trends in antibiotic exposure among infants in BC and then to estimate the concomitant changes in the burden of asthma that can be attributed to such trends.

2 Materials and methods

This study comprised of three major steps to achieve the objective: (1) ecological trend analysis of infant antibiotic exposure in BC, (2) meta-analysis of the dose-response relationship between infant antibiotic use and risk of childhood asthma, and (3) simulation modeling to quantify asthma burden under counterfactual scenarios regarding infant antibiotic exposure. The study period for which antibiotic exposure was quantified and asthma outcomes were modeled was 2001–2018.

All the analyses were conducted using R (version: 4.3.0) (21), Julia (version: 1.9.0) (22), and Stan (2.21) (23). The aggregated data and code used for the analyses are available in the GitHub repository: https://github.com/tyhlee/ImpactInfantAbxAsthma. This study was approved by the institutional review board of the University of British Columbia, Vancouver (H09–00650).

2.1 Ecological trend analysis of antibiotic exposure among infants in BC

We analyzed antibiotic use among infants (<1 year of age) using the population-based administrative health databases of BC from January 2001 (earliest year allowed in the simulation model) to December 2018 (last year of data availability for this study). In particular, the PharmaNet database contains dispensed medication records across community pharmacies and hospital outpatient pharmacies for all residents of the province (24). Medications dispensed to inpatients within hospitals or emergency departments are not included within this database. However, such prescriptions are less likely to be misused (and thus are unlikely to be affected by any policy targeting antibiotic exposure reduction), and are typically short-term. Data were extracted, anonymized, and made available to researchers by the BCCDC [Do Bugs Need Drugs? (H09-00650)]. All inferences, opinions and conclusions drawn in this study are those of the authors and do not reflect the opinions or policies of the Data Steward(s).

We computed the rate of infant antibiotic prescriptions per 1,000 infants using the population estimates from Statistics British Columbia (20). To estimate sex-specific trends, we fitted a negative binomial regression model for the log of the prescription rate, with calendar time and biological sex as covariates. An earlier study indicated a distinct decline in antibiotic use in 2005, coinciding with the introduction of a provincial antibiotic stewardship program (25). To account for

this non-linearity, we also included main-effect and interaction-effect terms between time and an indicator for whether the year was after 2005. We measured the goodness-of-fit of the model with the percentage deviance explained (the higher deviance explained the better, analogous to R^2 in an ordinary linear model, with values close to 100% justifying the use of regression-smoothed values instead of raw frequencies) (26).

2.2 Meta-analysis of the dose-response relationship between infant antibiotic use and childhood asthma prevalence

The analysis aimed to generate an equation that would map antibiotic exposure to asthma prevalence based on published information. We started with 63 studies that were identified in the systematic review by Duong et al. (8) which reported on the relationship between antibiotic use in early life and childhood asthma during the publication period between 1998 and 2021. We performed a further literature review (January 2021–September 2023) to identify any relevant studies published afterwards (details of the search are provided in Supplementary Materials Section 1; 13 studies were identified).

Using the 76 identified studies, we estimated the dose-response relationship between infant antibiotic use and childhood asthma prevalence (in terms of odds ratio). We applied the following inclusion criteria to the identified studies: (i) the dose-response relationship was investigated as part of an original research (not synthesized from other studies), (ii) the timing of antibiotic use was in the first year of life, (iii) the age range for asthma diagnosis was narrower than 6 years (if asthma diagnosis is made in children of ages between 3 and 6, then the age range is 4 years) and (iv) the risk of bias score was low [the overall risk of bias less than 2 based on the risk of bias domains of ROBINS-I (27) following the guidelines by the Agency for Healthcare Research and Quality (28)].

We made several assumptions and simplifications to enable the quantitative pooling of results. Specifically, some studies reported the association for a single year of age, while others documented the association over an age bracket. Based on dedicated simulation studies (see Supplementary Materials Section 2), we concluded that the effect size reported across an age range of less than 6 years would provide a good approximation for the single-year effect at the mid-point of the age range [studies reporting on longer ranges were thus excluded, see criterion (iii) above]. Second, we treated hazard ratios and odds ratios interchangeably, which is an acceptable approximation when event prevalence is low (29). Further, we assumed that the dose-response association was proportional on the logit scale (this assumption was qualitatively evaluated based on comparing the predicted vs. observed association for each study). Next, we classified the number of antibiotic prescriptions (of any days supply) as 0, 1, 2, 3, 4, and 5+. As some studies reported the associations for the number of antibiotic prescriptions as a range, we used the following categorization by taking the lower bound of the range: 1–2 prescriptions were recategorized as 1, and 3–4 prescriptions were recategorized as 3. Lastly, the included studies did not report on the interaction effect with sex, and existing evidence on this topic is weak (30); correspondingly, we assumed that the effect of infant antibiotic use was not different between males and females.

We fitted a random-effects meta-regression model with age of asthma diagnosis and infant antibiotic use as covariates in a Bayesian framework (31) (details on the model, implementation in Stan, and prior specification are provided in Supplementary Materials Section 3). The final equation was of the form:

$$\begin{aligned}&\text{Logit}(\text{asthma prevalence}|\text{age, dose})\\&= \beta_0 + \beta_{\text{Canada}} + \beta_{\text{age}} * \text{age} + \beta_{\text{dose}} * \text{dose},\end{aligned} \quad (1)$$

where age is the age of asthma diagnosis in years, dose is the number of antibiotic prescripts (0, 1, …, 4, 5+), β_0, β_{age}, β_{dose} are the fixed effects estimated by the meta-regression and β_{Canada} is the random effect corresponding to the single Canadian study in that analysis. This model specification enables pooling of all available evidence while also putting more weight on the single Canadian study.

2.3 Asthma policy simulation model

We used the Lifetime Exposures and Asthma outcomes Projection (LEAP) model, an asthma policy simulation model for Canada, to simulate counterfactual scenarios. Following the best practice recommendations in policy modeling (32), the LEAP model was developed, independently of this study, in collaboration with a steering committee of economic modelers, allergists, and respirologists across Canada. Model design, including important risk factors and the pathway of asthma, was informed from multiple rounds of Delphi processes to achieve consensus.

Details of this process and the model are reported elsewhere (33, 34). In brief, the LEAP model is an open-population discrete-time microsimulation platform that simulates a virtual Canadian population and follows it from birth or immigration to emigration, death, or the end of the pre-specified time horizon. After initializing the population in the starting year (2001 in our case), simulated individuals enter the model by birth or immigration in subsequent years. In each year, the actions and behaviors of the individual are simulated based on pre-defined mathematical equations, and the attributes and disease characteristics of the individual are updated accordingly. LEAP models a multitude of population outcomes including asthma incidence, prevalence, and exacerbations.

The LEAP model has been calibrated to the Canadian population, mimicking the demographic and disease characteristics of the Canadian population. Asthma incidence and prevalence were modeled and calibrated using the Chronic Disease Registry (35), and asthma exacerbations were calibrated using the Hospital Morbidity Database (36). Further, we incorporated the equations for antibiotic exposure among infants and the effect of infant antibiotic exposure on asthma prevalence (developed in the previous sections) into the LEAP model and then calibrated the LEAP model. Of note, LEAP does not model the disease course of asthma for children under 3 years of age due to difficulty in reliably diagnosing asthma in this age group. Further details of the LEAP model are provided elsewhere (34).

2.4 Evaluation of counterfactual scenarios

To investigate the impact of the observed decline in antibiotic use in BC between 2001 and 2018, we compared the following "what-if" scenarios. Scenario 1 was the base (factual) scenario using the smoothed trends (from the negative binomial regression) fitted to the observed data. Scenario 2 was a counterfactual scenario that modeled flat trends where the antibiotic prescription rate remained the same as the smoothed values in 2001. Scenario 3 was another counterfactual scenario that modeled a decline in the antibiotic prescription rate that was half of the smoothed trends (i.e., for any given year, the antibiotic prescription rate was the average of those in Scenarios 1 and 2).

The model was run separately for each scenario for the entire pediatric population (<19 years of age) of BC (approximately 1 million people in 2018) for 100 times. For each scenario, the following outcomes were recorded: total person-years with asthma (accounting for both the number of individuals with asthma and the amount of time each person with asthma spends during the study period), cumulative asthma incidence, and cumulative asthma exacerbations. We reported the average values of these measures across the 100 runs along with Monte Carlo (MC) standard deviations (SD). The results were provided by the following age groups (in years) as well: 3–4, 5–9, 10–14, and 15–18. Additionally, for each counterfactual scenario (Scenarios 2 and 3), differences and relative changes in each of the outcomes compared with the base scenario (Scenario 1) were recorded.

3 Results

3.1 Trends in antibiotic use

Over the period from 2001 to 2018, there were 773,160 infants born in BC. During the same period, 404,675 prescriptions of antibiotics for infants were recorded, corresponding to 523 prescriptions per 1,000 infants. Male infants received more prescriptions (586 per 1,000) than female infants (457 per 1,000). From 2001 to 2018, the number of female and male infants grew by 10.2% and 8.1%, respectively, whereas the prescription rate decreased by 71.2% for females and 71.6% for males, indicating a substantial decline in antibiotic exposure, as depicted in Figure 1.

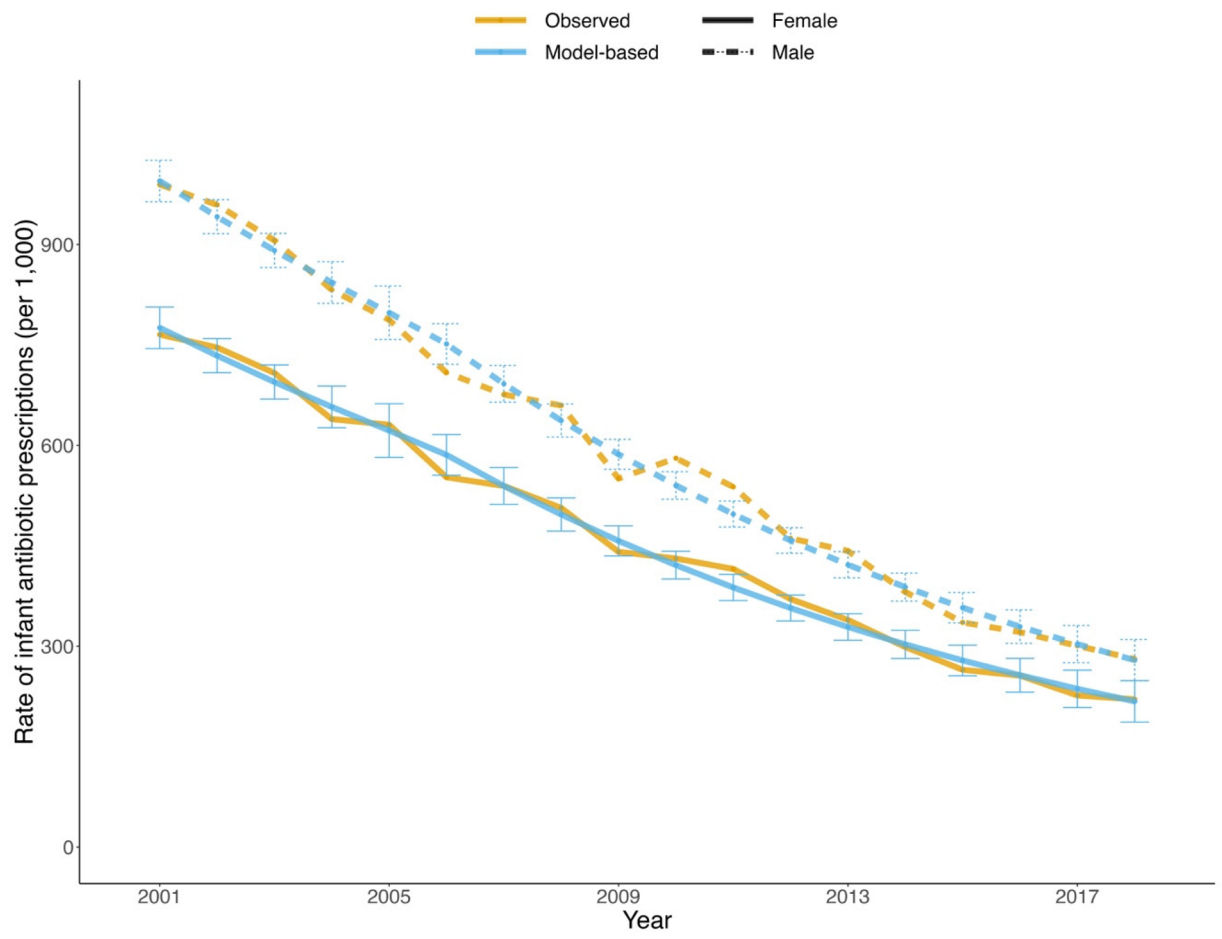

FIGURE 1
Observed (orange) and model-based (blue) trends in annual rate of infant antibiotic prescriptions by sex (solid: female; dotted: male) from 2001 to 2018 in British Columbia. The error bars indicate the 95% confidence intervals of the model-based values.

The negative binomial regression model fitted the data well with a goodness-of-fit of 99.3% deviance explained.

3.2 Dose-response relationship between infant antibiotic use and childhood asthma development

After examining the systematic review by Duong et al. (8) and the literature review of subsequent studies (Supplementary Materials Section 1), we found 6 studies that met our inclusion criteria [Figure 2 (37) and Supplementary Materials Section 3]. The studies were from Canada, Japan, Poland, South Korea, and the United States of America (11, 38–42). Publication years ranged from 2004 to 2020. The most recently published study reported on a prospective cohort in which asthma diagnosis was confirmed by a physician (11). One study reported on a cross-sectional cohort in which asthma diagnosis was based on self-report (41). The remaining four studies reported on retrospective cohorts in which asthma diagnosis was ascertained by international classification of disease (ICD-9 and ICD-10) codes (38–40, 42).

Figure 3 illustrates a dose-response relationship between the number of courses of antibiotics prescribed in the first year of life and asthma prevalence at different ages (3 years was the minimum age, as LEAP does not assign asthma attributes to children below this age) from the Bayesian meta-analysis (parameter estimates are provided in Table 1). There was a clear dose-response pattern, with the effect being higher with higher levels of antibiotic exposure (adjusted odds ratio of the dose effect: 1.05; 95% CI: 1.03–1.07) but attenuating over time (adjusted odds ratio of the ageing effect: 0.79; 95% CI: 0.77–0.82). Given the substantial attenuation at 7 years of age, we conservatively assumed the association is completely diminished beyond 7 years of age.

3.3 Population impact of counterfactual infant antibiotic exposure scenarios

The modeled antibiotic exposure trends by sex are presented for each scenario in Figure 4. The model produced asthma incidence and prevalence rates as expected under the base (factual) scenario (see Supplementary Materials Section 4). Furthermore, the decrease of 26.4% (MC SD: 0.2%) in asthma incidence among children of 1–4 years of age under the base scenario between 2001 and 2014 closely matched the reported value (26.0%) in the same age group in a similar period between 2000 and 2014 in BC in another independent study (11).

In Scenario 1 (base scenario), the model simulated an average (across 100 runs) of 1,982,861 (MC SD: 4,161) person-years with asthma, an average of 183,392 (MC SD: 417) cumulative asthma incident cases, and an average of 383,072 (MC SD: 1,129) cumulative exacerbations for the pediatric population (Table 2). In comparison, Scenario 2 resulted in estimated 1.9%–6.1%, depending on the outcome, relative increases in asthma outcomes

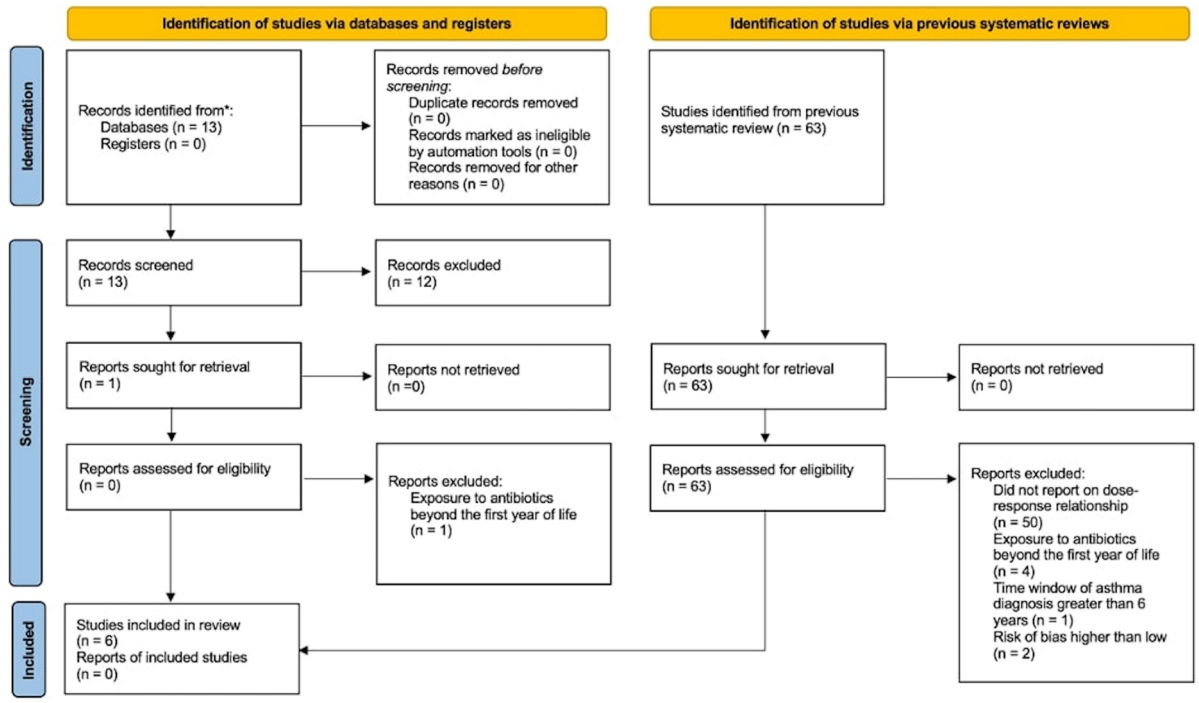

FIGURE 2
Flow diagram for meta-analysis. *MEDLINE Ovid was used to identify the records (see Supplementary Materials Section 3 for details).

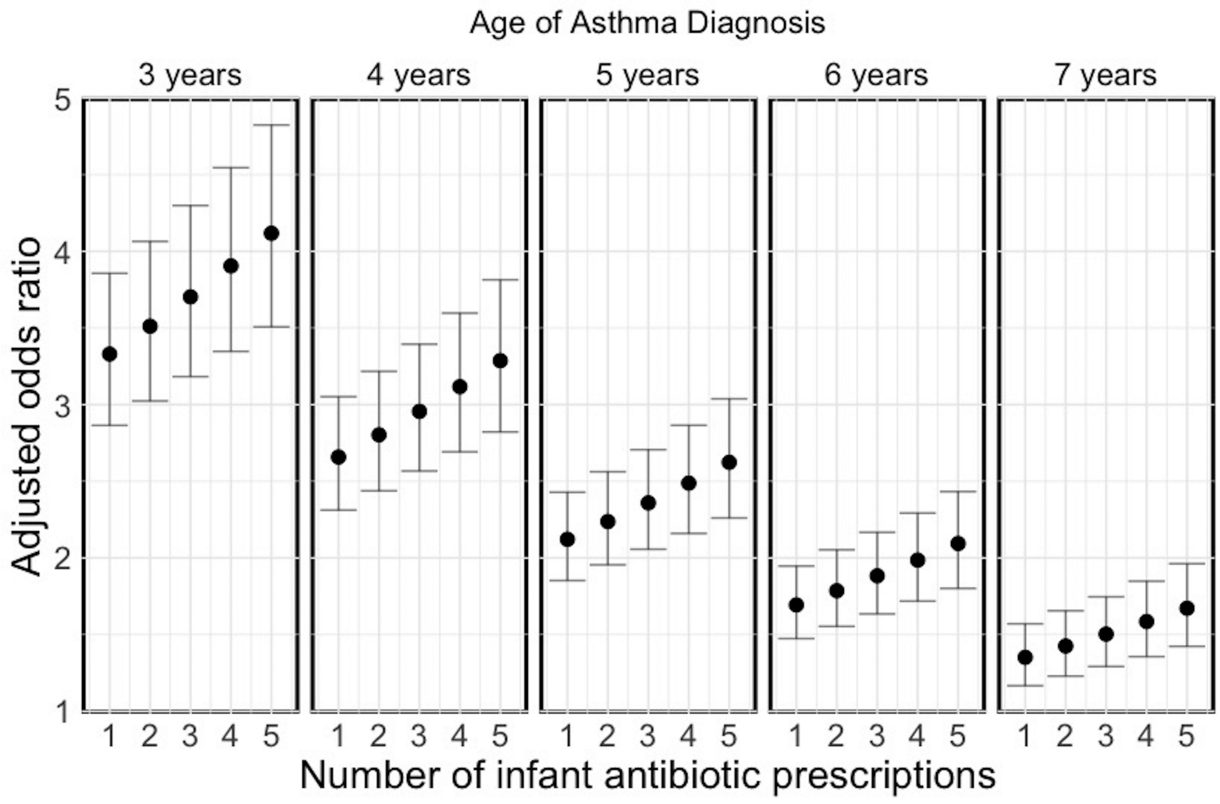

FIGURE 3
Dose-response relationship between infant antibiotic prescriptions in the first year of life and childhood asthma prevalence at different ages for Canada. The error bars indicate the 95% prediction intervals.

TABLE 1 Parameter estimates from the meta-regression of asthma prevalence.

Parameter	Estimate	95% credible interval
β_0	1.71	(0.77, 2.54)
β_{Canada}	0.12	(−0.69,1.06)
β_{age}	−0.23	(−0.26, −0.20)
β_{dose}	0.05	(0.03, 0.07)
$\sigma_u^{2\,a}$	0.93	(0.48, 1.99)

[a]σ_u^2 refers to the study-to-study variance component (see Supplementary Materials Section 3 for details).

during the study period. In absolute terms, if no decline in antibiotic exposure had happened, there would have been an additional 37,213 (MC SD: 5,370) person-years with asthma, 10,053 (MC SD: 558) asthma incident cases, and 23,280 (MC SD:1,455) pediatric exacerbations between 2001 and 2018 (Figure 5). The younger age groups (3–4 and 5–9) were responsible for the majority of the excess burden: 85.4% for cumulative person-years with asthma, 100.0% for cumulative asthma incident cases, and 98.2% for cumulative asthma exacerbations.

As expected, the relative increase in asthma outcomes (1.0%–3.3% depending on the outcome) was smaller for Scenario 2. In absolute terms, there were an additional 20,318 (MC SD: 5,119) person-years with asthma, 5,486 (MC SD: 515) asthma incident cases, and 12,728 (MC SD: 1,504) exacerbations during the study period compared to the base scenario (Table 2 and Figure 5). Similarly, the younger age groups (3–4 and 5–9) were mainly responsible for the excess burden: 84.3% for cumulative person-years with asthma, 100.0% for cumulative asthma incident cases, and 98.2% for cumulative asthma exacerbations.

4 Discussion

In this counterfactual population-level impact analysis, we used real-world trends from population-based data, quantitative evidence synthesis, Bayesian meta-analysis, and simulation modeling to quantify the population-level impact of changes in infant antibiotic exposure on the burden of asthma in a well-defined population over an eighteen-year period (2001–2018). The observed decline in antibiotic use was juxtaposed to two counterfactual scenarios, modeling the trends in antibiotic exposure in the first year of life that were different than the sustained decline observed during this period. We found that our projected results based on observed exposure trends closely matched the decrease in asthma incidence among children of 1–4 years of age between 2000 and 2014 in BC reported from a separate study (11), further validating the use of the policy model in our study. We estimated that if infant antibiotic use had not been reduced from 2001 levels, there would have been

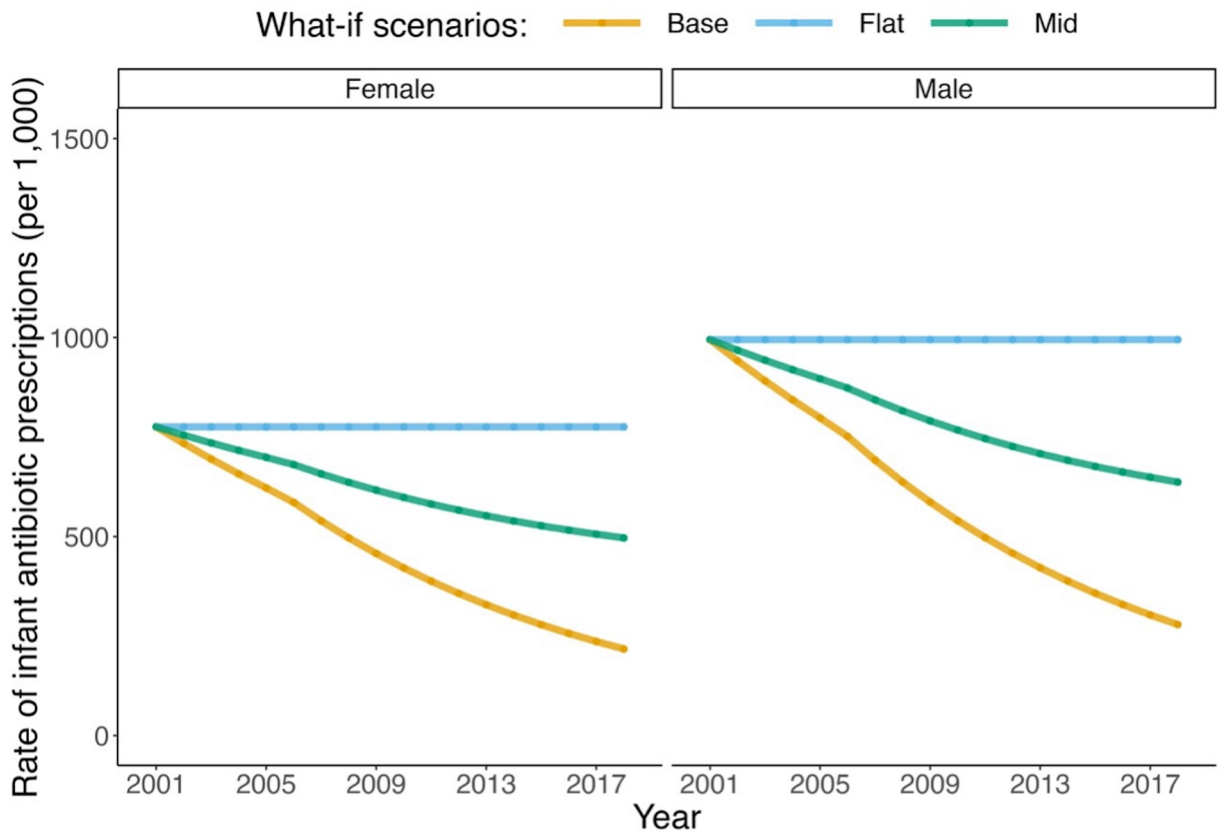

FIGURE 4
Trends in antibiotic exposure in infants for the three modeled scenarios. *Scenario 1 (base): smoothed trend fitted to observed data in the antibiotic prescription rate in British Columbia. *Scenario 2 (flat): trend in the prescription rate if it remained as the smoothed values in 2001. *Scenario 3 (mid): trend in the prescription rate if it declined by half of the smoothed trend.

excess 37,213 person-years with asthma in the pediatric population in this period. Putting this number in perspective, assuming annual direct medical costs of $550 (in 2024 Canadian dollars) for each person with asthma (43), BC has saved approximately $20 million on the burden of asthma in the pediatric population alone by reducing unnecessary use of antibiotics among infants during the study period (of note, the savings will higher if we include costs of antibiotic prescriptions).

These results can be seen as a prime example of far-reaching implications of reduction in antibiotic exposure in infants. Asthma is one of the manifestations of allergic diseases, whose risk is postulated to be affected by changes in gut microbiota (10). Considering the broader allergic conditions is likely to result in higher estimated benefit from reducing unnecessary antibiotic exposure. As well, the maximum age within which asthma outcomes were modeled in this study was 18 years (for infants born in 2001). For infants born later during the study period this time was shorter. A prevented case of asthma averts asthma-related burden throughout the life of the individual, translating to avoided exacerbations, reduction in quality of life due to symptoms, missed school days among children, and work productivity loss among adults for many years. As such, following the infant cohorts born within the study period into the future is likely to substantially increase the estimated benefits of antibiotic exposure reduction.

Already, without accounting for such benefits, interventions and policies aimed at reducing antibiotic exposure are found to be effective and most likely cost-saving (2, 44). A systematic review of 52 studies on the evaluation of antimicrobial stewardship programs revealed that they were associated with a 10% reduction in antibiotic prescriptions. The association was stronger for the low- and middle-income countries (an average decrease of 30%) and for pediatric patients (an average decrease of 21%), implying antimicrobial stewardship programs can be an effective intervention to deal with overuse of antibiotics. However, effectiveness alone, without considering costs, cannot guide decision-making. The Organization for Economic Co-operation and Development recently evaluated antimicrobial resistance control policies, including antimicrobial stewardship programs, across all its member countries (44). They used a microsimulation model that incorporated the impact of antibiotics on antimicrobial-resistant infections and modeled counterfactual scenarios to compare with a "no intervention" base scenario. They found that the stewardship programs would be cost-saving with a probability of at least 70%, cost-effective (at a threshold of US$50,000 per quality-adjusted life year) with a

TABLE 2 Evaluation of different trends in infant antibiotic use on asthma-related outcomes for the entire pediatric population (3–18 years of age) and by the age groups between 2001 and 2018.

Age group (years)	Estimated values*			Difference compared to Scenario 1		Relative change compared to Scenario 1 (%)	
	Scenario 1	Scenario 2	Scenario 3	Scenario 2	Scenario 3	Scenario 2	Scenario 3
Cumulative person-years with asthma							
3–4	115,339 (436)	128,577 (442)	122,560 (429)	13,238 (568)	7,220 (544)	11.5 (0.5)	6.3 (0.5)
5–9	526,680 (1,484)	545,222 (1,383)	536,593 (1,339)	18,542 (1,908)	9,913 (1,844)	3.5 (0.4)	1.9 (0.4)
10–14	729,025 (1,668)	734,116 (1,494)	731,875 (1,552)	5,091 (2,203)	2,851 (2,164)	0.7 (0.3)	0.4 (0.3)
15–18	611,817 (1,429)	612,159 (1,322)	612,150 (1,327)	343 (1,644)	334 (1,651)	0.06 (0.3)	0.1 (0.3)
Overall	1,982,861 (4,161)	2,020,074 (3,785)	2,003,178 (3,718)	37,213 (5,370)	20,318 (5,119)	1.9 (0.3)	1.0 (0.3)
Cumulative asthma incident cases							
3–4	79,976 (304)	89,362 (279)	85,110 (306)	9,387 (392)	5,134 (383)	11.7 (0.5)	6.4 (0.5)
5–9	62,621 (234)	63,301 (226)	62,983 (242)	680 (314)	362 (325)	1.1 (0.5)	0.6 (0.5)
10–14	24,914 (143)	24,897 (148)	24,896 (164)	−17 (187)	−18 (199)	−0.1 (0.8)	−0.1 (0.8)
15–18	15,882 (113)	15,885 (130)	15,889 (134)	3 (164)	8 (121)	0.0 (1.0)	0.1 (0.8)
Overall	183,392 (417)	193,445 (411)	188,878 (425)	10,053 (558)	5,486 (515)	5.5 (0.3)	3.0 (0.3)
Asthma exacerbations							
3–4	142,426 (659)	158,505 (608)	151,285 (715)	16,078 (886)	8,858 (887)	11.3 (0.7)	6.2 (0.6)
5–9	157,404 (601)	164,192 (634)	161,045 (651)	6,788 (845)	3,641 (877)	4.3 (0.6)	2.3 (0.6)
10–14	55,877 (300)	56,252 (270)	56,081 (276)	375 (360)	204 (387)	0.7 (0.7)	0.4 (0.7)
15–18	27,365 (176)	27,404 (157)	27,389 (192)	39 (216)	25 (256)	0.2 (0.8)	0.1 (0.9)
Overall	383,072 (1,129)	406,352 (1,130)	395,800 (1,172)	23,280 (1,455)	12,728 (1,504)	6.1 (0.4)	3.3 (0.4)

The values in the brackets are the Monte Carlo SD (across 100 runs).
*Average across 100 runs.
**Scenario 1 (base): fitted observed trends in the antibiotic prescription rate in British Columbia.
**Scenario 2 (flat): trends in the prescription rate that remained as observed in 2001.
**Scenario 3 (mid): trends in the prescription rate that declined by half of the fitted observed.

probability of at least 20%, and there was a <10% change of being not cost-effective. The chance of being cost-effective and cost-saving was likely underestimated, as the above-mentioned analysis did not consider the impact of antibiotic exposure on the burden of other diseases, such as asthma.

Ours was a simulation study on the benefit of reducing unnecessary antibiotic use on asthma-related outcomes based on the latest available evidence linking the two. In Canada, BC has the lowest antibiotic prescription rate among children. While this does not imply that BC has the lowest rate of unnecessary antibiotic prescriptions, the decrease in antibiotic use is attributed in part to an antimicrobial stewardship program in BC (25, 45). As such, this potentially indicates more room for improvement in other provinces and territories (46). This will help Canada break stagnant trends in asthma prevalence and severe asthma exacerbations requiring inpatient care (47, 48). Higher benefits are expected for many other jurisdictions, including low- and middle-income countries, many of which are observing an upward trend in antibiotic use among children under 5 years of age (49). Urgent initiatives involving collaboration among healthcare professionals, researchers, and policymakers are required to change the course of this trend and cope with a high proportion of overuse of antibiotics in both low- and middle-income countries and high-income countries (50, 51).

This study was not an analysis of any specific intervention for the reduction of antibiotic exposure. Instead, we focused on quantifying the current evidence on the association between antibiotic exposure and the population-level burden of asthma. Evaluating the outcomes of a specific intervention (e.g., implementing an antibiotic stewardship program in a jurisdiction) requires evidence on its resource implication, the effectiveness of the intervention on antibiotic use, as well as the multifaceted downstream implication of such change in the exposure. Dimensions of benefit from reducing unnecessary antibiotic use should be first studied in detail, as was done here for asthma, based on systematic and quantitative evidence synthesis and outcome modeling. Studies like ours, conducted for other conditions that might be affected by changes in antibiotic exposure, will provide evidence for a future meta-modeling study, collating all facets of the benefits and harms of exposure reduction initiatives. This will provide a complete picture of the effects of a specific intervention.

Our study has several strengths. Our estimation of infant antibiotic exposure was obtained from comprehensive, population-based data from the entire target population of a well-defined geographic area and health jurisdiction, without uncertainty due to sampling or bias due to representativeness. We used Bayesian meta-analysis to quantify high quality evidence from multiple different studies on the nuances of the dose-response association of primary interest. This is in contrast with a data-driven counterfactual analysis (which would have used the same data for both trend analysis of antibiotic exposure and estimation of dose-response association). The latter would restrict the evidence to one dataset. Further, given the nature of the data (administrative), controlling for potential confounding variables would have been difficult. Instead, we reconciled evidence on the trends observed from the population-based data with quantitative meta-analysis of all available evidence, and used computer modeling techniques to

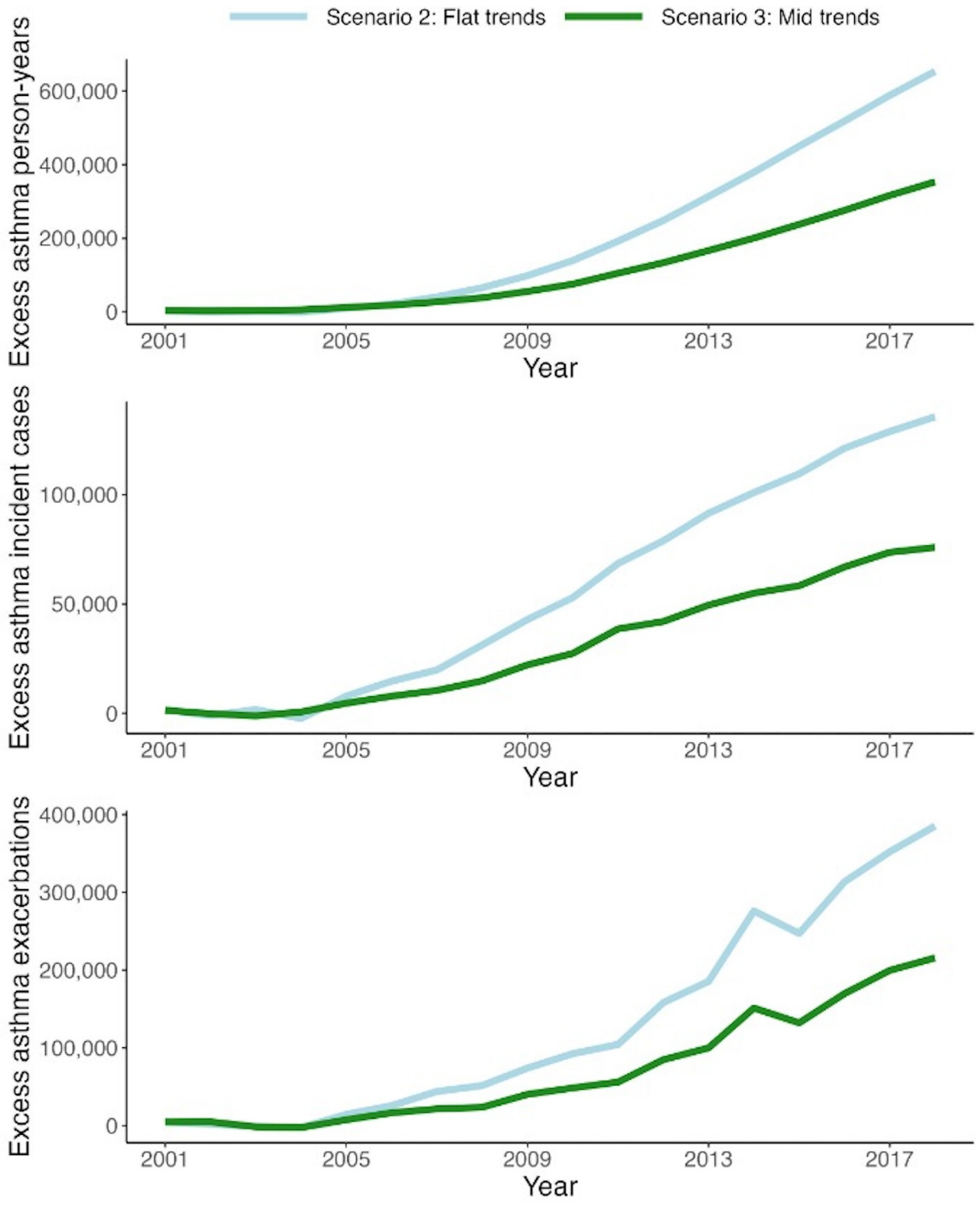

FIGURE 5
Excess number of asthma person-years (top), asthma incident cases (middle), and exacerbations (bottom) in the counterfactual scenarios (flat and mid) compared with the base scenario for the pediatric population (3–18 years of age). *Scenario 1 (base): smoothed trend fitted to observed data in the antibiotic prescription rate in British Columbia. *Scenario 2 (flat): trend in the prescription rate if it remained as the smoothed values in 2001. *Scenario 3 (mid): trend in the prescription rate if it declined by half of the smoothed trend.

propagate such evidence to change in asthma-related outcomes. Thus, simulation modeling allows for addressing the limitations of a single, static population-based study through evidence synthesis and various "what-if" scenarios (52). Of note, the policy model for asthma used in this work has been developed independently of this study (as a multi-purpose platform for policy analysis of asthma-related interventions in Canada) and has undergone rigorous analysis and independent validation studies (34).

The limitations of this study should also be acknowledged. A critical piece of evidence underlying the asthma model is the dose-response relationship between infant antibiotic use and childhood asthma prevalence that we estimated through the meta-analysis. We found a negative association that decayed over time (Figure 3). While the overall risk of bias was low in the included studies, the extent to which this association can be interpreted causally depends on the degree to which the included studies successfully controlled for confounding effects. In particular, potential biases due to confounding by indication or reverse causation were directly addressed in two of the included studies by excluding children who received antibiotics for respiratory symptoms or were diagnosed with asthma in the first year of life (11, 40). The remaining four studies could not fully account for confounding by indication or reverse causation (38, 39, 41, 42). We note that in our case, the original dose-response association of the Canadian study (11) was slightly attenuated after pooling evidence from other studies through the meta-analysis. Moreover, due to a lack of evidence in the literature, examining whether the dose-response relationship holds for antibiotic exposure beyond the first year of life is difficult. Evidence from mechanistic studies suggests that the critical window for gut microbiome maturation appears to be within the first 100 days of life (53, 54). Not modeling any effect beyond one year therefore seems justified. This conservative assumption means any effect beyond the first year of life will increase the benefits observed in this study. However, in general, more robust evidence is needed to better understand the causal relationship on early life antibiotic exposure and the risk of asthma, and the impact this relationship has when scaled up to large populations (55).

Another limitation is the difficulty in confirming asthma diagnosis in children under 6 years of age (56). Our decision to model asthma cases in that age group (specifically 3 years or older) was to reflect and incorporate current labeling of health utilization related to asthma by healthcare systems. In Canada, asthma cases in the administrative health databases are identified based on a validated case definition (57). Of note, misdiagnosis and remission are accounted for in the LEAP model in order to match asthma prevalence, which rises in early childhood and declines during adolescence (see Supplementary Figure 3). Regarding the meta-analysis, three included studies examined the asthma diagnosis in children under 6 years of age (11, 38, 42). Yoshida et al. used a conservative approach based on a typical diagnostic code case definition combined with confirmed use of ICS and controller medications, likely underestimating asthma cases (38). In contrast, in the studies by Patrick et al. (11) and Celedon et al. (42), asthma diagnosis was confirmed by physicians [which is the recommended approach by the Canadian Thoracic Society (58)]. These studies acknowledged the difficulty and potential misclassification of asthma diagnosis in young children despite their efforts. Last but not least, the LEAP model in its current implementation does not consider uncertainty in the evidence. Further, we did not evaluate costs for this study. It is obvious from our results that costs associated with antibiotic use and asthma will both decrease with reduced unnecessary antibiotic exposure (whereas costs will increase if necessary antibiotics are wrongly reduced).

There are several areas for future research. Modeling studies are required for other conditions that might be affected by antibiotic exposure. Specific programs and interventions aimed at reducing unnecessary antibiotic exposure should be evaluated in dedicated analyses, which should also incorporate costs to guide policymaking. Further, there is now robust evidence indicating that the effect of antibiotic use on allergic disease is mediated via the gut microbiota (10, 11). We note that another modifier of gut microbiota is breastmilk exposure (59, 60). Recent evidence suggests that breastmilk works as an effect-modifier between prenatal and infant antibiotic use and childhood asthma development (18, 61). Specifically, breastmilk exposure has been shown to largely mitigate the elevated risk of asthma posed by antibiotic exposure through enrichment with *Bifidobacterium longum subsp. infantis* (18). Subsequent modelling work in BC has shown that observed and expected childhood asthma incidences are very similar when taking both reduced population-level antibiotic exposure in infancy and increased breastfeeding rates into account (62). Therefore, a joint intervention targeting infant antibiotic overuse and promoting breastfeeding (particularly where antibiotic exposure is necessary in infancy) can lead to more effective prevention against childhood asthma development. The outcome of such policies should be investigated in the future.

In summary, this study quantified the far-reaching benefits of reducing unnecessary antibiotic use on the burden of asthma. Our findings present compelling evidence to urge jurisdictions with overuse of antibiotics in infants and high prevalence of childhood asthma to implement antimicrobial stewardship initiatives. Incorporating the life-long impact on the burden of asthma and potentially other diseases will increase the value of interventions addressing the overuse of antibiotics in infancy. Future work should incorporate utility and costs to provide a measure on the value-for-money potential of such interventions.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the institutional review board of the University of British Columbia,

Vancouver (H09–00650). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

TL: Data curation, Formal Analysis, Methodology, Software, Visualization, Writing – original draft. JP: Writing – review & editing, Conceptualization, Methodology, Supervision. AS: Writing – review & editing, Data curation. FM: Writing – review & editing, Conceptualization, Data curation. ST: Writing – review & editing. HL: Writing – review & editing, Conceptualization. DP: Writing – review & editing. JC: Writing – review & editing. KJ: Writing – review & editing. MS: Conceptualization, Methodology, Supervision, Writing – review & editing, Funding acquisition.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was funded by a research grant from the Canadian Institutes of Health Research and Genome Canada (274CHI). The funders had no role in any aspect of this study and were not aware of the results.

Acknowledgments

We would like to acknowledge the Community Antimicrobial Stewardship research team at BC CDC (http://www.bccdc.ca/our-services/programs/community-antimicrobial-stewardship) for their feedback and assistance with access to the data on antibiotic prescriptions. Access to data provided by the Data Stewards is subject to approval but can be requested for research projects through the Data Stewards or their designated service providers. The following data sets were used in this study: Chronic Disease Registry and Pharmanet. All inferences, opinions, and conclusions drawn in this publication are those of the author(s), and do not reflect the opinions or policies of the Data Steward(s).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/falgy.2024.1491985/full#supplementary-material

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frontiers | Frontiers in Allergy

TYPE Original Research
PUBLISHED 21 January 2025
DOI 10.3389/falgy.2024.1463867

OPEN ACCESS

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RECEIVED 12 July 2024
ACCEPTED 26 December 2024
PUBLISHED 21 January 2025

CITATION
Medeleanu MV, Reyna ME, Dai DLY, Winsor GL, Brinkman FSL, Verma R, Nugent E, Riaz N, Simons E, Mandhane PJ, Azad MB, Turvey SE, Moraes TJ and Subbarao P (2025) Exploring the potential mediating role of systemic antibiotics in the association between early-life lower respiratory tract infections and asthma at age 5 in the CHILD study. Front. Allergy 5:1463867. doi: 10.3389/falgy.2024.1463867

Exploring the potential mediating role of systemic antibiotics in the association between early-life lower respiratory tract infections and asthma at age 5 in the CHILD study

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Objective: Lower respiratory tract infections (LRTIs) in early life are one of the strongest risk factors for childhood asthma and are often treated with systemic antibiotics (IV or oral). We aimed to explore the association between early-life LRTIs and systemic antibiotics on asthma development and the potential mediating role of antibiotics in this relationship.

Methods: Data were collected as part of the longitudinal, general Canadian population CHILD Study. LRTIs during the first 18 months of life were identified through parental symptom report at regular study visits. Systemic antibiotic use was defined as at least one dose of oral/intravenous antibiotics between birth and the 18-month visit and were further categorized by indication as either given for a respiratory indication (upper or lower respiratory symptoms) or non-respiratory indication. Asthma was diagnosed by in-study pediatricians at the 5-year study visit. Adjusted logistic regression models and mediation analyses via systemic antibiotics use were performed.

Results: Among 2,073 participants included in our analysis, 72 (4.9%) had asthma age 5, and 609 (29.3%) used systemic antibiotics before the 18-month visit. Among children who had taken antibiotics, 61.6% also had an LRTI in that period compared to 49.7% among children without exposure to systemic antibiotics ($p < .001$). Moderate-severe LRTIs before age 18 months were associated with higher odds of 5-year asthma [aOR 4.12 (95%CI 2.04–7.95) $p < .001$]. Antibiotics taken for respiratory indications were associated with

higher odds of asthma at age 5 [aOR 2.36 (95%CI 1.59–3.48) $p < .001$]. Children who received systemic antibiotics for only non-respiratory indications during the first 18 months of life were not associated with increased odds of asthma [aOR 1.08 (95%CI 0.44–2.30) $p = .851$]. Using mediation analysis, 21.7% of the association between LRTI and asthma is estimated to be mediated through use of early-life systemic antibiotics. However, a significant direct effect of moderate-to-severe LRTIs on asthma risk remained in adjusted mediation models ($p = .014$).

Conclusion: Through mediation modeling we estimate that the increased risk of asthma at age 5 that is associated with moderate-severe LRTIs in infancy may be partially mediated by systemic antibiotics taken during the first 18 months of life. This underscores the importance of public health strategies focused on antibiotic stewardship and reducing early life LRTIs to mitigate asthma risk.

KEYWORDS

preschool asthma, respiratory tract infections, antibiotics, mediation analyses, cohort study, clinical epidemiology

Introduction

Asthma is one of the most common chronic diseases among children in the United States and Canada and is characterized by reversible airway obstruction, airway hyper-responsiveness, and airway inflammation (1–3). Lower respiratory tract infections (LRTIs) in the first years of life are one of the strongest risk factors for childhood asthma (4, 5) with recent meta-analyses estimating that children who experienced a LRTI in infancy are up to three times more likely to develop wheezing in adolescence and that this association persists into adulthood (6). Given that by age two years about 80% of children are exposed to a respiratory virus, it is imperative to disentangle the underlying mechanisms and modifiable risk factors driving these associations (7). Recent interest has pointed to analyzing modifiable factors in early life such as the use of systemic antibiotics in the treatment of LRTI.

Antibiotic use in the first year of life has consistently been reported to be strongly associated with later asthma diagnosis (8–12). Moreover, investigators reported that at a population level in British Columbia, Canada, each 10% increase in antibiotic prescriptions under age 1 was associated with a 24% increase in asthma prevalence between 1 and 4 years of age (11). Other studies suggest that the indication for antibiotic prescription may affect the risk of asthma development, noting that systemic antibiotics prescribed to treat respiratory infections (e.g., amoxicillin, penicillin, cephalosporin, and macrolides) resulted in a greater risk of asthma compared to systemic antibiotics prescribed for non-respiratory indications, such as a urinary tract infection (10). Antibiotics taken for non-respiratory infections have also been associated with asthma development but with lower estimated effects (10, 13).

Despite consistent associations between LRTIs in early life and the development of later asthma, their independent or joint effect with early-life antibiotic use has not been thoroughly studied. Specifically, it is unclear whether early-life systemic antibiotics serve as a mediating variable of the association of early life LRTIs and subsequent childhood asthma. In the present study, we analyze data from the longitudinal multi-center CHILD Study to assess the relationship between symptomatic LRTIs in the first 18 months of life and physician-diagnosed asthma by 5 years of age, and to estimate the mediating role of antibiotics use up to 18 months of age.

Methods

Study population

The CHILD Study enrolled pregnant mothers during their second or third trimester from the general Canadian population, with an initial recruitment of 3,624 mothers between 2008 and 2012 (14). After healthy delivery at 35 weeks gestation or later, 3,454 mothers remained eligible. The study, spanning four locations across Canada (Vancouver, Winnipeg and Morden-Winkler, Edmonton, and Toronto) and had inclusion criteria of mother's age being >18 years (>19 years in Vancouver), residence within 50 km of the delivery hospital and had consented to cord blood donation. Exclusion criteria covered major congenital abnormalities, respiratory distress syndrome, multiple births, plans to relocate within a year, *in vitro* fertilization-conceived children, and those not spending more than 80% of their time at their primary listed home.

Lower respiratory tract infections

Parent/caregiver reported history of colds and respiratory symptoms from birth to 18 months of age were collected at regular study intervals (3-, 6-, 12-, and 18-month study visits). We developed a symptom-based LRTI definition after a thorough review of the literature regarding epidemiological definitions of respiratory infections in children [detailed in (15)]. An LRTI was defined as the presence within the last 3 or 6 months of: (i) a

cold and (ii) a fever, and (iii) a cough, chest congestion or trouble breathing (Supplementary Figure 1). LRTI severity was classified as mild, or moderate/severe based on health care utilization. Moderate-severe infections were any LRTI that required an unscheduled doctor visit (moderate), a hospital visit or an emergency department visit (severe). Children could have an LRTI with or without wheeze symptoms, as it was not required for our definition to minimize the risk of identifying children already showing potential signs of propensity to asthma (airway hyperresponsiveness).

Given that clinical diagnosis and/or virological testing for all LRTIs was unavailable, we performed validation of our symptom-based LRTI using a subset of CHILD Study participants (365 families) using Viral Score Cards modified from the URECA Wisconsin Upper Respiratory Symptom Survey for Kids (WURSS-K) and nasal swabs for PCR sequencing of nine viruses (Influenza A, Influenza B, Respiratory syncytial virus, Rhinovirus, Enterovirus, Parainfluenza 1, 2 and 3, Metapneumovirus and Adenovirus) (16). Parents/caregivers were asked to call study investigators and fill out the URECA WURSS-K Viral Score Cards by phone during every period of respiratory illness in the first year of life. Infants then were scored as having no symptoms (score = 0), mild symptoms (score 1–3), moderate symptoms (score = 4), or severe symptoms (score $\geq$5). Infants who received moderate or severe scores underwent nasal swabs that were subsequently PCR sequenced for nine viruses including Influenza A, Influenza B, Respiratory syncytial virus, Rhinovirus/Enterovirus, Parainfluenza 1,2 and 3, Metapneumovirus and Adenovirus. Symptoms associated with positive viral swabs are detailed in Supplementary Table 1. Symptoms of "runny nose", "wiping nose once per hour", "cough in the last three days" and "fever" were most prevalent among positive viral swabs (Supplementary Table 1). We performed a receiver operating characteristic (ROC) analysis to identify the diagnostic performance of our LRTI and URTI definitions in this subset of CHILD participants. Any positive viral swab was assigned as true positive reference.

LRTI definition validation

In our LRTI definition validation sub-study, 365 participants notified the study team of a "cold". 70% of these colds were assigned "mild" scores while 13% of kids were assigned "severe" scores. Moderate to severe "colds" occurred in 24% of sub-study who then underwent nasal swabs ($n = 86$). Enterovirus/Rhinovirus (ER) (25.58%) and RSV (16.26%) were the most common agents among the positive swabs. A minority were positive for *S.pneumonia* (11.63%) and 13.95% were negative for the entire panel tested (Supplementary Figure 1). In our AUROC analysis, an LRTI definition that included fever had the highest area under the curve (AUC = 0.70) for a positive swab compared to the LRTI definition without fever (AUC = 0.52) suggesting that including fever significantly improved detection of LRTI cases and this definition was therefore used in all subsequent analyses (Supplementary Figure 2, Table 2).

Antibiotic medication histories

Antibiotics used between birth and the 18-month visit were obtained from CHILD Study medication questionnaires completed by a parent or caregiver at the 3-,6-,12-,18-month visit. Children were classified as exposed or unexposed to early life systemic antibiotics if at least one oral/intravenous antibiotic course was reported between birth and 18-month visit. Early-life systemic antibiotic use from hereon refers to at least one dose of oral/intravenous antibiotics between birth and the 18-month visit.

Antibiotic use was further classified as used for any respiratory or only non-respiratory indications. Children who received systemic antibiotics for any respiratory indications [upper respiratory tract infections, ear infection (otitis media), sinusitis, sore throat, croup, bronchiolitis, bronchitis, pneumonia, combinations of cold, congestion, cough and fever symptoms, influenza, and respiratory distress at birth] were included in the respiratory indication group. Children who only received antibiotics for non-respiratory indications (eye infections, sepsis, urinary tract infections, skin conditions such as eczema, rash, hives, and impetigo or various fungal infections) were included in the non-respiratory indication group. Children who received a systemic antibiotic for both a respiratory and non-respiratory infection were included in the any respiratory antibiotic group. Similarly, if a child received multiple antibiotics during infancy for both reasons, they were included in the any respiratory antibiotic group.

To control for the possibility that prior exposure to antibiotics may affect the risk of early LRTI, participants who received systemic antibiotics at a visit prior to when they reported their first LRTI were excluded from analyses. Finally, to avoid confounding by reverse causation, children who received systemic antibiotics due to wheeze or asthma symptoms were excluded from analyses.

Asthma diagnosis and wheezing

Pediatric asthma specialists conducted structured interviews with accompanying parent or guardian at in-person clinic visits to identify symptoms and physical findings consistent with asthma (14). Study physicians answered the question: "In your opinion, does this child have asthma? (Yes/Possible/No)". Children in the "Possible" category were considered as "No asthma".

Covariates

Child ethnicity (Caucasian/non-Caucasian), parity (older siblings yes/no), household annual income ($0–$49,999 CAD, $50,000–$99,999, $100,000–$149,999, >$150,000), maternal and paternal physician-diagnosed asthma (ever/never diagnosed), and any prenatal smoke exposure (yes/no) were self-reported at enrollment through questionnaires during the second or third trimester of pregnancy. Infant sex (female/male), gestational age

and weight at birth and mode of delivery (cesarian/vaginal) were obtained from birth records. Information regarding the duration (months) and exclusivity (exclusive or partial/none) of breastfeeding in the first 3, 6 and 12 months were collected through repeated questionnaires within the first two years of life. Information on time spent away from home at 18 months was defined as significant time (>7 h per week, yes/no) spent away from home, and was collected through parent questionnaires. Information on recurrent wheezing was collected through clinical assessments performed at the 1-year and 3-year visits. At the clinical visit, parents were asked if the child had a wheezing noise coming from their chest in the past 12 months (yes/no). Recurrent wheezing was defined as 2 or more episodes of wheeze in the last 12 months reported at the 3-, 6- or 12 month CHILD Health Questionnaires.

Statistical methods

Descriptive statistics are presented as mean (standard deviation, SD) for continuous measures, and frequency (%) for categorical measures. *P*-values were obtained by *t*-test, Fisher's exact test and chi-square test where appropriate.

Adjusted logistic regression analyses were performed using the R *stats* package "glm" function to estimate the odds ratios (OR) and 95% confidence intervals (CI) for the association of any LRTI in early life, LRTI severity (No, Mild, Moderate-Severe) and systemic antibiotic use (No vs. Yes) with asthma diagnosis at 5 years of age. Each exposure was analyzed separately and in combined models to determine their relationships with 5-year asthma development. All models were adjusted by study site, child sex, ethnicity (Caucasian), breastfeeding status at 3-months, prenatal smoke exposure, mode of delivery, family income, time spent away from home at 12-months, presence of older siblings, and parental history of asthma. To explore the impact of indication, we replicated our logistic regression models in children with antibiotics taken for a respiratory indication (any respiratory antibiotic use vs. no systemic antibiotics) and a second set examining the impact of non-respiratory antibiotics (non-respiratory antibiotic use vs. no systemic antibiotics).

Mediation analyses were performed using the Baron & Kenny approach in the R *mediation* package "mediate" function using a mediator model assessing the direct relationship between LRTI severity and antibiotic use, and an outcome model evaluating the effect of these factors on 5-year asthma. To satisfy the binary exposure required in mediation analysis, moderate-severe infections were compared to a combined reference group of children who reported mild or no LRTIs in the first 18 months. The total, direct, and indirect effects (mediation) were estimated, with adjustments made for potential confounders. Confidence intervals were calculated via bootstrapping ($n = 1{,}000$ simulations). Mediation analysis was also undertaken after excluding children with antibiotics due to non-respiratory indications.

Finally, a sensitivity analysis using the same set of confounders as the primary analysis was performed in non-wheezing children to investigate if the effects remained consistent in children not predisposed to asthma. R version 4.2.2 was used to perform all analyses.

Results

Out of all participants in the CHILD Study ($N = 3{,}454$), 3,301 children had at least one respiratory infection questionnaire completed during the first 18 months of life. Among participants with respiratory infection questionnaires available, 2,073 (62.8%) also had an available history of antibiotic medication between birth and 18-month visit (at least two antibiotic questionnaires competed) along with an asthma diagnosis recorded at the 5-year visit (Supplementary Figure 2). These participants were included in subsequent analyses. Of these participants ($n = 2{,}073$), 52% were male, 65% were Caucasian, 16.5% had prenatal smoke exposure and 37% had a family history of asthma (Table 1). 1,420 (43.0%) of these participants reported at least one LRTI in the first 18 months (Supplementary Table 3, Supplementary Figure 5). Prevalence of any LRTI or most severe LRTI during the first 18 months of life in the CHLD Study are provided in Supplementary Figure 5.

LRTI and antibiotic Use

1,464 of the 2,073 included participants reported no systemic (oral or IV) antibiotics taken in the first 18 months of life and 609 (29.4%) reported taking 1 or more dose of systemic antibiotics during this period. Of these participants, 132 (6.4%) reported antibiotics taken for only non-respiratory indications and 477 (23.0%) reported taking antibiotics for any respiratory indication (Table 1).

Participants who reported an LRTI in the first 18 months of life were more likely to have older siblings (56.6% of Moderate-severe, 47.0% of Mild vs. 38.0% of No LRTI group), more recurrent wheezing (32.9% Moderate-severe vs. 81.8% Mild vs. 5.6% of the No LRTI group), and more 3-year and 5-year asthma (Table 1). Participants who received systemic antibiotics were significantly more likely to be male (57.6 vs. 51.9%), be from the Toronto or Winnipeg study sites ($p = .009$), significantly more likely to have early and more frequent LRTIs in the first 18 months than children with no systemic antibiotics use (Table 1). These children were also significantly more likely to spend time away from home at 18-months (63.3% vs. 55.0%), have older siblings (47.5% vs. 41.3%), not be exclusively breastfed at hospital discharge, 3- or 6-months ($p = .038, .052, .073$), and had higher rates of 1- and 3-year recurrent wheeze (8.3% vs. 6.0% and 8.4% vs. 6.0%), and 3- and 5-year physician diagnosis of asthma (8.9% vs. 4.6% $p < .001$, 9.4% vs. 4.9%) (Table 1). Compared to children without systemic antibiotics, participants with antibiotics taken for a respiratory indication were significantly more likely to have LRTIs in the first 18 months, had higher weight for age z-score at birth, higher weight at the 5-year visit, spent more time away from home at 18-months, were more likely to have older siblings, had higher rates of 1- and 3-year recurrent wheeze (14.7% vs. 6.0% and 13.5% vs. 7.5%), and 3- and 5-year

TABLE 1 Demographic and clinical characteristics of CHILD study participants by history of LRTI or systemic antibiotics taken between birth and 18-month visit. All *p*-value comparisons made to "No LRTI" or "No systemic antibiotics" in the first 18 months group (*n* = 2,073).

	Lower respiratory tract infections (LRTI)				Systemic antibiotics						
	No LRTI	Mild LRTI	Moderate-Severe	*p*	No systemic antibiotics	Any systemic antibiotics	*p*	Non-respiratory indication	*p*	Respiratory indication	*p*
	(*n* = 971)	(*n* = 1,026)	(*n* = 76)		(*n* = 1,464)	(*n* = 609)		(*n* = 132)		(*n* = 477)	
Sex assigned at birth, male (%)	499 (51.4)	572 (55.8)	40 (52.6)	.146	760 (51.9)	351 (57.6)	**.020**	80 (60.6)	.068	271 (56.8)	.070
Caucasian, yes (%)	647 (66.6)	677 (66.0)	47 (61.8)	.690	955 (65.2)	416 (68.3)	.195	85 (64.4)	.922	331 (69.4)	.107
Study Site (%)				.221			**.009**		.807		**.006**
Edmonton	145 (14.9)	176 (17.2)	13 (17.1)		241 (16.5)	93 (15.3)		22 (16.7)		71 (14.9)	
Toronto	251 (25.8)	219 (21.3)	20 (26.3)		340 (23.2)	150 (24.6)		30 (22.7)		120 (25.2)	
Vancouver	222 (22.9)	260 (25.3)	20 (26.3)		381 (26.0)	121 (19.9)		30 (22.7)		91 (19.1)	
Winnipeg	353 (36.4)	371 (36.2)	23 (30.3)		502 (34.3)	245 (40.2)		50 (37.9)		195 (40.9)	
LRTI in the first 18 months	–	–	–	–	728 (49.7)	375 (61.6)	**<.001**	60 (45.5)	.396	315 (66.0)	**<.001**
Age of the first LRTI	–	11.87 (4.14)	10.15 (4.41)	–	11.99 (4.20)	11.24 (4.10)	**.009**	10.28 (4.30)	**.009**	11.39 (4.06)	**.048**
Total LRTI in the first 18 months				–			**.026**		.286		**.041**
1 LRTI reported	–	652 (77.3)	36 (48.0)		465 (78.2)	224 (69.1)		31 (68.9)		193 (69.2)	
2 LRTI reported	–	168 (19.9)	25 (33.3)		110 (18.5)	83 (25.6)		11 (24.4)		72 (25.8)	
3 LRTI reported	–	19 (2.3)	11 (14.7)		16 (2.7)	14 (4.3)		3 (6.7)		11 (3.9)	
4 LRTI reported	–	4 (0.5)	3 (4.0)		4 (0.7)	3 (0.9)		0 (0.0)		3 (1.1)	
Weight for age z-score at birth [mean (SD)]	0.30 (1.00)	0.32 (0.96)	0.29 (0.82)	.84	0.30 (0.96)	0.33 (1.01)	.483	0.08 (1.05)	**.014**	0.40 (0.99)	**.046**
Weight at 3-year, kg [mean (SD)]	15.12 (1.98)	14.98 (1.74)	15.01 (1.86)	.288	15.01 (1.86)	15.13 (1.89)	.178	14.94 (1.70)	.661	15.19 (1.94)	.076
Height at 3-year, kg [mean (SD)]	96.03 (4.01)	95.81 (3.85)	95.97 (4.08)	.481	95.85 (3.88)	96.07 (4.06)	.269	95.47 (4.11)	.285	96.23 (4.04)	.071
Weight at 5-year, kg [mean (SD)]	19.64 (2.98)	19.41 (2.76)	19.28 (2.34)	.136	19.43 (2.79)	19.72 (2.98)	**.031**	19.42 (2.94)	.996	19.80 (2.98)	**.011**
Height at 5-year, kg [mean (SD)]	110.80 (4.77)	110.68 (4.70)	110.49 (4.59)	.769	110.68 (4.66)	110.87 (4.88)	.388	110.18 (5.15)	.252	111.06 (4.79)	.118
Prenatal smoke exposure, yes (%)	166 (17.1)	161 (15.7)	12 (15.8)	.69	242 (16.5)	97 (15.9)	.785	18 (13.6)	.460	79 (16.6)	1.00
Prenatal annual family income, CAD (%)				.741			.623		.358		.821
$0–$49,999	105 (10.8)	114 (11.1)	12 (15.8)		164 (11.2)	67 (11.0)		14 (10.6)		53 (11.1)	
$50,000–$99,999	305 (31.4)	314 (30.6)	23 (30.3)		468 (32.0)	174 (28.6)		34 (25.8)		140 (29.4)	
$100,000–$149,999	251 (25.8)	276 (26.9)	14 (18.4)		374 (25.5)	167 (27.4)		44 (33.3)		123 (25.8)	
> $150,000	215 (22.1)	230 (22.4)	21 (27.6)		323 (22.1)	143 (23.5)		28 (21.2)		115 (24.1)	
Prefer not to say	95 (9.8)	92 (9.0)	6 (7.9)		135 (9.2)	58 (9.5)		12 (9.1)		46 (9.6)	
Time away from home <18 m, yes (%)	434 (55.4)	515 (59.1)	42 (63.6)	.19	654 (55.0)	337 (63.3)	**.001**	60 (56.1)	.911	277 (65.2)	**<.001**
Older siblings, yes (%)	369 (38.0)	482 (47.0)	43 (56.6)	**<.001**	605 (41.3)	289 (47.5)	**.012**	62 (47.0)	.243	227 (47.6)	**.019**
Delivery mode, cesarian section (%)	233 (24.0)	251 (24.5)	18 (23.7)	.965	343 (23.4)	159 (26.1)	.215	43 (32.6)	**.025**	116 (24.3)	.738
Hospital breastfeeding status				.359			**.038**		**.004**		.312
Exclusive	622 (74.3)	664 (76.4)	51 (79.7)		963 (77.2)	374 (71.5)		71 (63.4)		303 (73.7)	
Partial	197 (23.5)	184 (21.2)	10 (15.6)		256 (20.5)	135 (25.8)		36 (32.1)		99 (24.1)	
None	18 (2.2)	21 (2.4)	3 (4.7)		28 (2.2)	14 (2.7)		5 (4.5)		9 (2.2)	

(*Continued*)

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TABLE 1 Continued

	Lower respiratory tract infections (LRTI)				Systemic antibiotics						
	No LRTI	Mild LRTI	Moderate-Severe	*p*	No systemic antibiotics	Any systemic antibiotics	*p*	Non-respiratory indication	*p*	Respiratory indication	*p*
	(*n* = 971)	(*n* = 1,026)	(*n* = 76)		(*n* = 1,464)	(*n* = 609)		(*n* = 132)		(*n* = 477)	
3-month breastfeeding status				.130			.052		.159		.130
Exclusive	614 (63.2)	627 (61.1)	41 (53.9)		925 (63.2)	357 (58.6)		77 (58.3)		280 (58.7)	
Partial	234 (24.1)	284 (27.7)	21 (27.6)		376 (25.7)	163 (26.8)		33 (25.0)		130 (27.3)	
None	123 (12.7)	115 (11.2)	14 (18.4)		163 (11.1)	89 (14.6)		22 (16.7)		67 (14.0)	
6-month breastfeeding status				.584			.073		.466		.113
Exclusive	178 (18.4)	205 (20.0)	12 (15.8)		292 (20.0)	103 (16.9)		22 (16.8)		81 (17.0)	
Partial	592 (61.2)	603 (58.9)	44 (57.9)		878 (60.2)	361 (59.4)		78 (59.5)		283 (59.3)	
None	197 (20.4)	216 (21.1)	20 (26.3)		289 (19.8))	144 (23.7)		31 (23.7)		113 (23.7)	
Inhalant sensitization at 1-year, yes (%)	35 (3.7)	39 (3.9)	3 (4.1)	.981	55 (3.8)	22 (3.7)	.992	3 (2.3)	.623*	19 (4.1)	.919
Atopy 1 year, yes (%)	120 (12.8)	126 (12.5)	10 (13.5)	.956	188 (13.1)	68 (11.5)	.345	18 (14.1)	.868	50 (10.8)	.208
Atopy 3 year, yes (%)	126 (13.7)	141 (14.3)	10 (14.3)	.918	187 (13.3)	90 (15.7)	.202	21 (16.7)	.364	69 (15.4)	.315
Atopy 5 year, yes (%)	180 (19.4)	193 (19.5)	18 (24.3)	.582	262 (18.7)	129 (21.8)	.121	30 (23.1)	.270	99 (21.5)	.213
Recurrent wheezing 1-year, Yes (%)	54 (5.6)	90 (8.8)	25 (32.9)	**<.001**	88 (6.0)	81 (13.3)	**<.001**	11 (8.3)	.385	70 (14.7)	**<.001**
Recurrent wheezing 3-year, Yes (%)	73 (7.6)	93 (9.2)	17 (22.4)	**<.001**	108 (7.5)	75 (12.4)	**<.001**	11 (8.4)	.832	64 (13.5)	**<.001**
Asthma 3-year, yes (%)	48 (5.1)	56 (5.6)	15 (20.5)	**<.001**	66 (4.6)	53 (8.9)	**<.001**	6 (4.6)	1.00*	47 (10.2)	**<.001**
Asthma 5-year, yes (%)	48 (4.9)	67 (6.5)	14 (18.4)	**<.001**	72 (4.9)	57 (9.4)	**<.001**	7 (5.3)	.833*	50 (10.5)	**<.001**
Parental history of asthma, Yes (%)	356 (36.7)	385 (37.5)	36 (47.4)	.178	538 (36.7)	239 (39.2)	.308	39 (29.5)	.120	200 (41.9)	**.049**

Bold indicates significant *p* values.

*Fisher's exact test was used instead of Chi-square testing for any categorical variables where $n < 5$.

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physician diagnosis of asthma (10.2 and 10.5% vs. 4.6 and 4.9%) (Table 1). Children who received systemic antibiotics for a respiratory indication by the 18-month visit had significantly higher rates of parental history of asthma compared to the no systemic antibiotics group (41.9% vs. 36.7%) (Table 1).

LRTI and systemic antibiotics in the first 18 months are associated with asthma at 5-years

Participants who reported a LRTI in the first 18 months of life had greater odds of 5-year asthma (aOR 1.50 [95%CI 1.04–2.20] $p = .033$ ($n = 1{,}103/2{,}073$) (Figure 1). Children with moderate-severe LRTIs had significantly higher odds of 5-year asthma [aOR 4.12 (95%CI 2.04–7.95) $p < .001$] ($n = 76/2{,}073$) (Figure 1). Children with only mild LRTIs in the first 18 months of life had no significant increase in odds of 5-year asthma compared to the no LRTI group [aOR 1.34 (95%CI 0.91–1.98) $p = .145$] ($n = 1{,}026/2{,}073$) (Figure 1).

Systemic antibiotics taken by the 18-month visit was associated with a significantly increased adjusted odds ratio of 5-year asthma [2.07 (95%CI 1.43–3.01) $p < .001$] ($n = 609/2{,}073$) (Figure 1). In particular, systemic antibiotics taken for a respiratory indication (upper or lower respiratory) were associated with higher odds of asthma at the 5-year visit (aOR 2.36 [95%CI 1.59–3.48] $p < .001$ ($n = 477/1{,}941$) (Figure 1). However, children who received systemic antibiotics for only non-respiratory indications by 18 months were not associated with increased odds of asthma [aOR 1.08 (95%CI 0.44–2.30) $p = .851$] ($n = 132/1{,}596$).

Next, we explored the association between severity of LRTI and history of early-life antibiotics. We observed that a higher portion of participants with a history of moderate-severe LRTI in the first 18 months had received systemic antibiotics during that period (73%) compared to mild (30.6%) and no LRTI participants (23.7%) (Figure 2A). Children with moderate-severe infections (defined here as requiring unscheduled healthcare services) more often received systemic antibiotics during early life, both for non-respiratory indications (only) (16.7% vs. Mild; 7.3% and No; 8.9%) (Figure 2B) as well as for respiratory indications (72% of Moderate-Severe LRTI participants vs. 27.11% of Mild and 18.02% of No LRTI) (Figure 2C).

Antibiotics mediate the relationship between moderate-severe LRTI and 5-year asthma

We next explored the combined associations between LRTIs and systemic antibiotics during the first 18 months of life on 5-year asthma diagnosis. LRTI under 18 months were significantly associated with systemic antibiotics taken during that period [aOR 1.60 (95%CI 1.32–1.95) $p < .001$](Table 2). In combined models of any LRTI and any systemic antibiotics under 18 months, only antibiotics were significantly associated with increased odds of 5-year asthma [aOR 2.00 (95%CI 1.37–2.90) $p = .002$] (Table 2). Since LRTIs were not significantly associated with asthma in combined models with antibiotics ($p = .08$) (Table 2), mediation analyses focused on moderate-severe infections.

In combined mutually adjusted models of LRTI severity and any systemic antibiotics taken before the 18-month visit, both moderate-to-severe LRTIs [aOR 3.36 (95%CI 1.59–6.81) $p = .001$] and systemic antibiotics were significantly associated with 5-year asthma [aOR 1.82 (95%CI 1.21–2.72) $p = .004$] (Table 2). Since both moderate-severe LRTI and systemic antibiotics were associated with increased risk of asthma, we explored the potential mediating effect of systemic antibiotics taken for any indication.

In our first mediation analysis we estimated that 21.7% ($p = .004$) of the association between moderate-to-severe LRTIs and 5-year visit asthma was mediated through use of systemic antibiotics [$\beta_{\text{indirect effect}} = 0.025$ (95%CI 0.008–0.050), $p = .002$] ($n = 2{,}070$) (Table 3, Figure 3). A direct effect of moderate-to-severe LRTIs on 5-year asthma remained significant in this model [$\beta_{\text{direct effect}} = 0.091$ (95%CI 0.0019–0.170), $p = .014$] (Table 3, Figure 3).

Indication for antibiotics and early-life recurrent wheeze alter the mediating role of systemic antibiotics on asthma

We observed a stronger association between 5-year asthma and early systemic antibiotics used for respiratory indications (Figure 1) and therefore replicated our mediation models after stratifying participants by indication. First, we examined the mediating role of antibiotics taken for a respiratory indication on the association between early-life LRTIs and 5-year asthma. Similar associations for moderate-severe LRTI [aOR 2.85 (95%CI 1.33–5.82), $p = .005$] and systemic antibiotics for any respiratory indication remained with 5-year asthma [aOR 2.05 (95%CI 1.36–3.08), $p = .001$] among children who received antibiotics for any respiratory indication ($n = 1{,}941$) (Supplementary Table 4). Significant mediation of the association between moderate-severe LRTIs and 5-year asthma (27.7%, $p = .006$) remained among children who took systemic antibiotics for any respiratory indications [$\beta_{\text{indirect effect}} = 0.031$ (95%CI 0.015–0.050), $p = .002$] (Supplementary Table 5). A significant direct effect of moderate-severe LRTI on risk of 5-year asthma remained among these participants [$\beta_{\text{direct effect}} = 0.081$ (95%CI 0.011–0.160), $p = .026$] (Supplementary Table 5).

Next, we explored whether the association between systemic antibiotics for a respiratory indication and asthma may be an artefact of children with pre-existing wheeze symptoms. We performed a sensitivity mediation in a subset of $n = 1{,}748$ participants who did not have recurrent wheezing already present at the 1-year visit (Supplementary Table 6, Figure 3). Mediation analysis in non-wheezers estimated that 36.3% ($p = .030$) of the relationship between moderate-to-severe LRTIs before 18 months and asthma at age 5 was mediated through systemic antibiotics taken for a respiratory indication [$\beta_{\text{indirect}} = 0.039$ (95%CI 0.015–0.060), $p < .001$]. However, there was no significant direct effect of moderate-severe LRTI in the non-recurrent wheezing subset

Model	Factor level	n	aOR [95%CI] p-value
LRTI reported by 18 months	*Yes vs. None*	1103/2073	1.50 [1.04 – 2.20] p=.033
LRTI Severity reported by 18 months of age	*Mild LRTI vs. None*	1027/2073	1.34 [0.91 – 1.98] p=.145
	Moderate-Severe LRTI vs. None	76/2073	4.12 [2.04 – 7.95] p<.001
Any systemic antibiotics taken by the 18-month visit	*Any systemic antibiotic vs. None*	609/2073	2.07 [1.43 – 3.01] p<.001
Systemic antibiotics for a respiratory indication before the 18-month visit	*Respiratory indication vs. None*	477/1941	2.36 [1.59 – 3.48] p<.001
Systemic antibiotics for only non-respiratory indications before the 18-month visit	*Non-respiratory indication vs. None*	132/1596	1.08 [0.44 – 2.30] p=.851

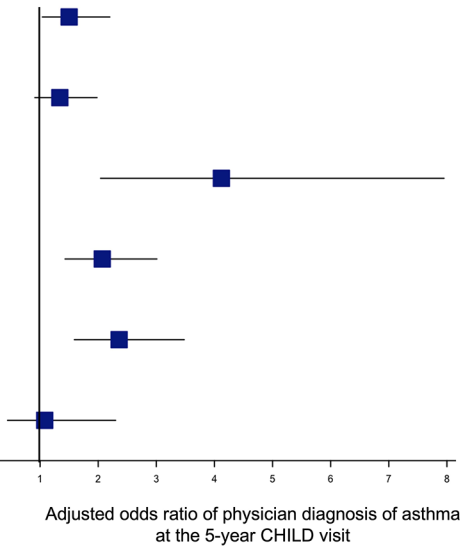

FIGURE 1
Adjusted odds ratios (OR) and 95% confidence intervals (CI) of physician diagnosis of asthma at 5 years of age for LRTIs and antibiotics between birth and 18-month visit. Models adjusted by site, child sex, Caucasian, breastfeeding <3 m, prenatal smoke exposure, mode of delivery, family income, away from home <12 m, older siblings, and parental history of asthma.

[$\beta_{direct} = 0.069$ (95%CI −0.015 - 0.160), $p = .110$] (Supplementary Table 7, Figure 3).

Finally, systemic antibiotics used between birth and 18 months for only non-respiratory indications was not associated to 5-year asthma in this analysis [aOR 1.08 (95%CI 0.44–2.30), $p = .851$] (Supplementary Table 7). Mediation analysis in this subset of participants was not pursued due to the limited number of participants with moderate-severe LRTI and antibiotics taken in infancy for only non-respiratory indications ($n = 8$).

Discussion

Using data collected in the CHILD Study we investigated the role of LRTIs and systemic antibiotics in the first 18-months of life on asthma diagnosis at the 5-year visit. While in our adjusted models the presence of any LRTI in the first 18 months was not a significant risk factor for asthma, we found that moderate-severe LRTIs significantly increased the estimated risk of asthma at age 5. Through mediation analyses we estimated that over 20% of this association (moderate-severe LRTIs and asthma) may be attributed to systemic antibiotic exposure, particularly among children who were prescribed antibiotics for a respiratory indication. Moreover, these associations were present among children without wheezing symptoms in the first year of life and preceding symptoms of early life asthma.

We provide evidence that systemic antibiotics taken in the first 18 months of life, regardless of the indication, partially mediates the association between moderate-severe LRTI and 5-year asthma. In subsequent mediation analyses, we identified that the proportion mediated was increased in cases where the systemic antibiotics were taken for respiratory indications. Suggesting a direct association between infection severity on asthma, independent of, yet compounded by, antibiotic use. In our sensitivity analysis in only non-recurrent wheezers (excluding children with recurrent wheezing at 1-year visit), the mediating effect of respiratory antibiotics was increased even further, with no significant direct effect of moderate-severe LRTIs remaining. These results suggest that in children without early symptoms of asthma, a substantial proportion of the association between moderate-severe LRTIs and asthma may be explained by systemic antibiotic exposure rather than a respiratory infection itself.

Our study is the first to investigate the mediating role of antibiotic use in the association between early-life LRTIs and asthma at age 5. Nonetheless, our results support observations from other studies. Bentouhami et al. (17) performed an incidence density study nested in a data collection project with information on 1,128 mother–child pairs where systemic antibiotic use in the first year of life was defined as excessive (≥4 courses) vs. non-excessive (<4 courses) use based on information from weekly diaries (17). The authors found a stronger association between the incidence of asthma and the use of systemic antibiotics in the first year of life among children who had LRTIs (defined as having had bronchitis with or without chronic cough and/or pneumonia according to the reporting of the parents) during that time. The asthma incidence density ratio (IDR) was significantly higher for children with LRTIs (IDR 5.17)

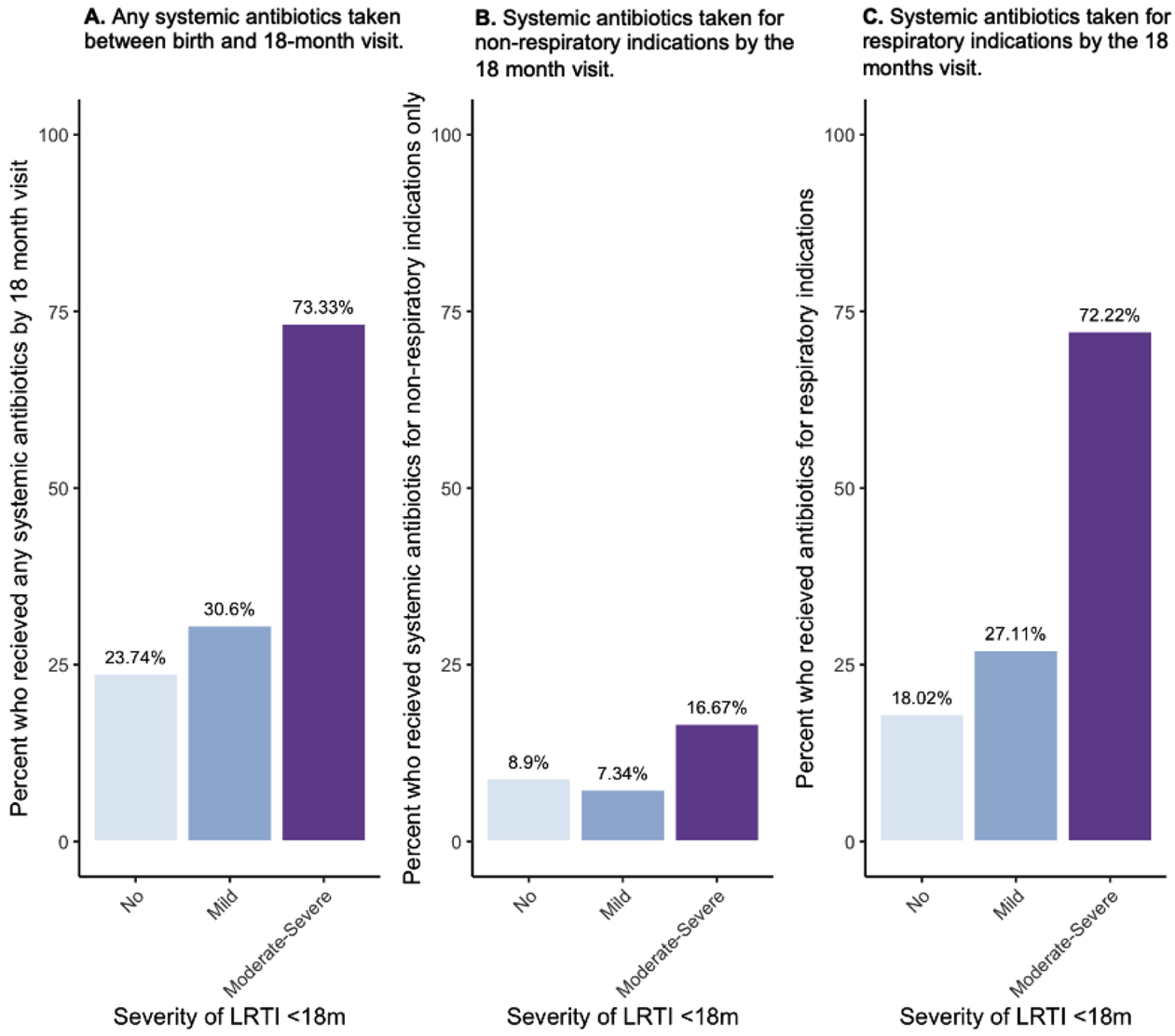

FIGURE 2
Percent of CHILD study participants who had taken systemic antibiotics between birth and the 18-month visit by indication (any reason, only non-respiratory, any respiratory) and by severity of LRTIs reported by 18 months of age. (A) Percent (%) of participants who received any systemic antibiotic across LRTI severities, (B) percent of participants who took antibiotics due to only non-respiratory indications across LRTI severities, (C) percent of participants who took antibiotics by the 18-month visit due to respiratory indications by LRTI severity group.

compared to those without (IDR 1.49). Similarly, the Longitudinal Study of Australian Children (LSAC) found that antibiotics given to children between birth and 24 months increased their risk of developing asthma later in childhood (6 and 15 years old) using medication record data (18). After LSAC authors accounted for respiratory infections that prompted antibiotic use (such as ear infections, hospitalization for fever or viral infections in the first year of life), early antibiotic use in children still significantly increased the risk of developing early-persistent asthma by 2.3 times (95% CI: 1.47–3.67, $p < 0.001$) compared to those without antibiotic exposure (18). Finally, a systematic review demonstrated that after adjusting for respiratory infections, there was still a significant and independent association between antibiotics and asthma (although decreasing from OR 1.38 to OR 1.16 after adjustment) (19).

Several mechanisms have been proposed to explain the association between LRTIs, antibiotics, and asthma. One hypothesis suggests that antibiotics may eliminate beneficial bacteria, which otherwise have a protective effect, thereby increasing the risk of allergic illnesses. Another possibility is that antibiotics with anti-inflammatory properties, such as macrolides, may inhibit type 1 immune responses, leading to a dominance of type 2 immune responses. Antibiotics might also contribute to the development of asthma by disrupting the human microbiome in a critical period when the human microbiome and immune system are developing (20–22). It is hypothesized that this effect is caused through perturbations in the populations, community succession, and diversity of the airway and gut microbiota. Using >2 antibiotic treatments between birth and 11 months of age was associated with an increased risk of asthma which was partially

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TABLE 2 Individual and combined regression models analyzing the association between any LRTIs within the first 18 months of life (LRTI <18 m) or the severity of those LRTIs (LRTI severity) and the use of systemic antibiotics for any indication before the 18-month visit, on the odds of an asthma diagnosis at age 5.

	Adjusted LRTI <18 m model[a] (*N* = 2,073)			Adjusted LRTI severity model[a] (*N* = 2,073)			
	aOR	CI	*p*		aOR	CI	*p*
Crude models: asthma 5Y~X							
Asthma 5Y~LRTI < 18 m				Asthma 5Y~LRTI severity			
LRTI < 18 m				LRTI severity			
Yes	1.50	1.04–2.20	**0.033**	Mild	1.34	0.91–1.98	0.145
–				Moderate-severe	4.12	2.04–7.95	**<0.001**
Asthma 5Y~1 + antibiotics				Asthma 5Y–1 + antibiotics			
Any systemic antibiotics	2.07	1.43–3.01	**<0.001**	Any Systemic Antibiotics	1.87	1.26–2.74	**0.002**
Mediator model: antibiotics (M)~LRTI <18 m(X)				Mediator model: antibiotics (M)~LRTI severity (X)			
Antibiotics~1 + LRTI < 18 m				Antibiotics~1 + LRTI severity			
LRTI < 18 m				LRTI severity			
Yes	1.60	1.32–1.95	**<0.001**	Mild	1.41	1.15–1.72	**0.001**
–				Moderate-severe	9.05	5.37–15.85	**<0.001**
Outcome model: 5-year asthma (Y)~antibiotics (M) + LRTI < 18 m (X)				Outcome model: 5-year asthma (Y)~antibiotics (M) + LRTI severity (X)			
LRTI < 18 m				LRTI severity			
Yes	1.40	0.96–2.06	0.080	Mild	1.29	0.86–1.95	0.216
–				Moderate-severe	3.36	1.59–6.81	**0.001**
Any antibiotics	2.00	1.37–2.90	**<0.001**	Any antibiotics	1.82	1.21–2.72	**0.004**

Bold indicates significant *p* values.

[a]Models adjusted by: site, child sex, Caucasian, breastfeeding <3 m, prenatal smoke exposure, mode of delivery, family income, away from home <12 m, older siblings, and parental history of asthma.

TABLE 3 Mediation 1: systemic antibiotics taken for any indication as a mediator between severe LRTI and 5-year asthma (*N* = 2,070).

Adjusted model[a] (*N* = 2,070)				
	OR (95% CI)			*p*-value
5-year asthma[a]~X				
Moderate-to-severe LRTI (vs. mild & no)	3.51 (1.79, 6.46)			**<.001**
Any systemic antibiotics	2.05 (1.4, 2.97)			**<.001**
Mediator model: systemic antibiotics by 18 months ~LRTI severity				
Moderate-to-severe LRTI	7.58 (4.54, 13.15)			**<.001**
Outcome model: asthma 5Y~LRTI severity + systemic antibiotics by 18 months				
Moderate-to-severe LRTI	2.79 (1.39, 5.26)			**.002**
Any systemic antibiotics	1.83 (1.24, 2.68)			**.002**
Mediation output	β	95% CI lower	95% CI upper	*p*
Total effect	0.117	0.039	0.210	**.002**
Indirect effect (ab)	0.025	0.008	0.050	**.002**
Direct effect (c')	0.091	0.019	0.170	**.014**
Proportion mediated,%	21.7%	7.7	54.0	**.004**

Bold indicates significant *p* values.

[a]Models adjusted by: site, child sex, Caucasian, breastfeeding <3 m, prenatal smoke exposure, mode of delivery, family income, away from home <12 m, older siblings, and parental history of asthma.

mediated through longitudinal change in the composition of the nasal microbiome (12). Infant antibiotic use was associated with elevated *Moraxella, Haemophilus,* and *Streptococcus,* which are pathogenic associated genus (23) and high *Moraxella sp.* abundance is associated with preschool asthma (12, 24). There is also evidence that antibiotics increase the risk of asthma through their influence on the gut microbiome (25–27) thought to occur through depletion of beneficial bacteria with fermentative capacity (e.g., short chain fatty acid producing species), decreased overall diversity and therefore reduced resistance to pathogenic bacteria colonization (28). These microbial disruptions can exacerbate airway inflammation or may impact proper immune system development increasing the risk of developing asthma. In the CHILD Study, the number of antibiotics courses within the first year of life was associated with decreased gut alpha diversity, and this effect was greatest when taken under 3 months of age (11). CHILD authors further demonstrated that the gut microbiome diversity at 1 year mediated the relationship between infant antibiotic use and asthma diagnosis at age 5 (11).

In our analyses we followed the most widely used mediation methodology outlined by Baron and Kenny (11) which requires the following assumptions to be satisfied: (i) no misspecification of causal order, (ii) no misspecification of causal direction, (iii) no misspecification due to unmeasured variables, and (iv) no misspecification due to imperfect measurement (29). By measuring and defining our variables in terms of temporality and having a theory for biological plausibility we minimize risks of violating the first two assumptions. However, as an observational study it is not possible to guarantee complete adherence of the latter two assumptions. We acknowledge that our reliance on

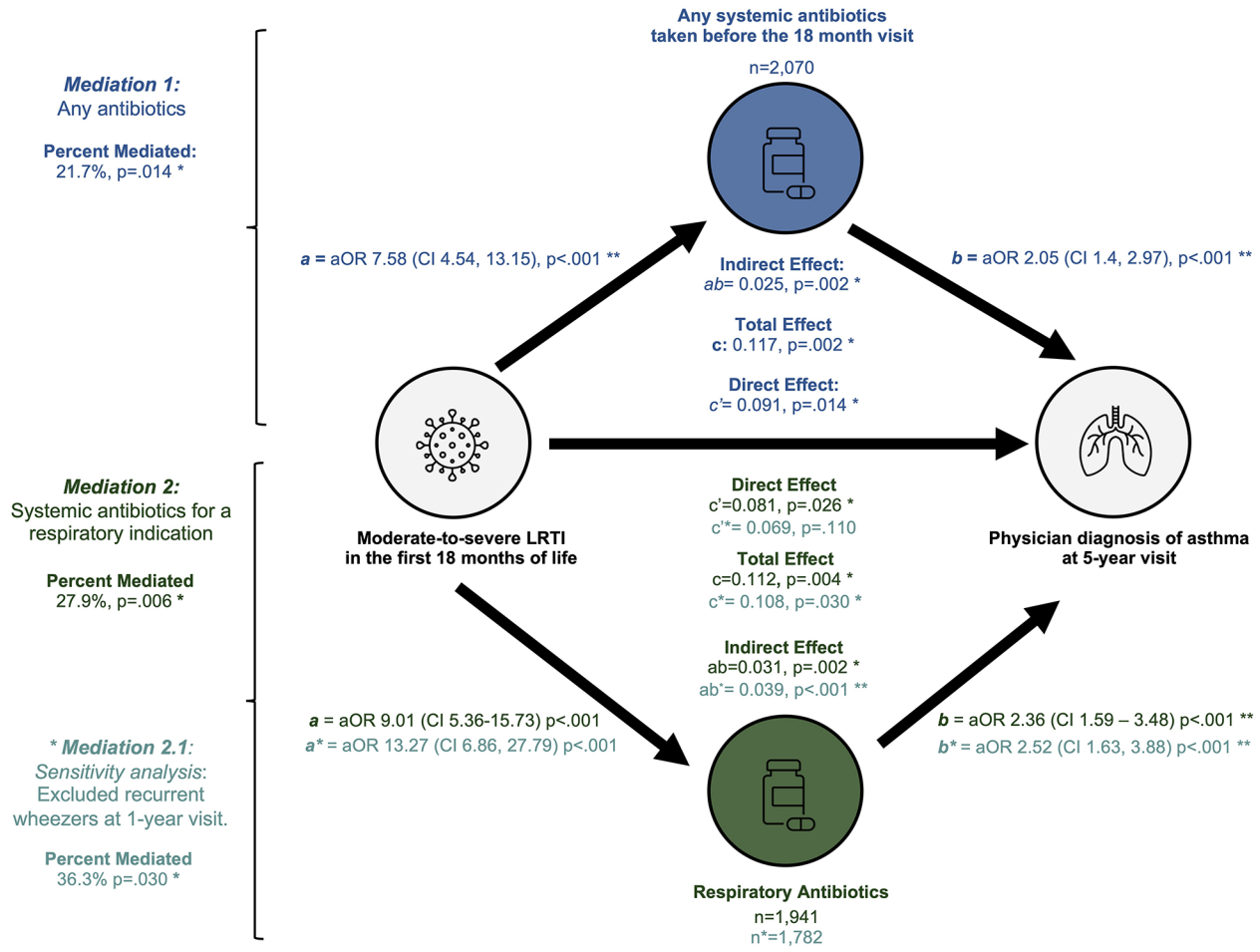

FIGURE 3
Summary of mediation analyses. This figure illustrates the three mediation models performed: any systemic antibiotics (top), antibiotics for any respiratory indication (second from bottom) and respiratory indication among non-wheezers (bottom).

parental reporting may overestimate LRTI prevalence. We attempted to mitigate this by including fever as a required factor in our symptom-based definition of LRTIs. Fever is an objective indicator of symptomatic infection that parents can measure; inclusion of fever in our symptom-based definition is supported by our validation analysis of LRTI symptoms vs. virological testing, where including fever significantly improved the identification of true positives (laboratory confirmed LRTIs). The trade-off between the increased specificity of objective diagnostic tests and decreased applicability in primary care or low-resource settings needs to be evaluated in the design of future research studies.

In this context, our classification of infection severity using healthcare utilization may also be biased by parental behavior. Parents with heightened vigilance due to increased medical literacy or family history of asthma may be more inclined to seek medical care. Such patterns could influence our classification of LRTI severity and the likelihood of receiving antibiotics. However, our use of a validated LRTI definition ensures that the classification is based on consistent, clinically relevant criteria rather than subjective or behavioral factors, improving the reliability of our assessment. Additionally, we attempted to minimize these parental factors by adjusting our models for family income and family history of asthma. Our symptom-based LRTI definition may be used in future population-based studies to explore the associations reported here and could be adapted for other parental reported studies, data-linkage studies, or in low-income settings where objective testing for infection may not be readily available.

Given that our LRTI and antibiotic data rely on parental reports collected through questionnaires administered at regular intervals, we could not report on associations outside these intervals, such as the impact of earlier or later timing of infections or antibiotics during the first 18 months, or the cumulative antibiotic exposure in infancy. Nonetheless, findings from a smaller subset of CHILD Study participants that linked administrative data found a significant dose-response between number of antibiotic courses and asthma, with other studies reporting similar findings (11, 18, 30). While we were unable to replicate this approach in our mediation models due to sample size limitations, this gap highlights an area for future investigation, particularly concerning the hypothesis that earlier-

timing of infections and frequent antibiotic treatment could be more detrimental to the development of the microbiome and immune system. Imperfect measurement of asthma in this age group may also be possible. Differentiating early signs of asthma from symptoms of early respiratory infections is a complex methodological challenge. In young children, asthma is often used as an umbrella diagnosis that is not well defined. As a result, the classification of pediatric asthma may be susceptible to bias, with certain subtypes potentially being underrepresented or overrepresented.

Another limitation to our mediation methodology is that any interactions between LRTIs and type of antibiotics cannot be tested. We attempted to explore this question using an alternative approach, by conducting stratified analysis by antibiotic indication (31). Among children who had received respiratory antibiotics only, we estimated a significant mediating effect of 21.7%. Unfortunately, we were unable to replicate this analysis in children who had received only non-respiratory antibiotics due to insufficient sample size in this mediator group, a challenge that would have persisted in a formal interaction analysis. This limited sample size likely reflects the high co-occurrence of LRTIs and antibiotics during this period, where many LRTIs were treated with antibiotics. As the CHILD Study is a general population cohort, the small number of participants with moderate-to-severe LRTIs treated exclusively with non-respiratory antibiotics further constrained our analysis. Additionally, our classification of antibiotics into "any respiratory" and "non-respiratory" indications complicates categorization, as children may receive antibiotics for multiple indications. To address this, and in line with our primary objective, we included children who received antibiotics for both indications in the "any respiratory antibiotic" group, capturing instances where antibiotics for LRTI could mediate the respiratory pathway to asthma. However, interpreting these findings requires caution, as attributing risk solely to respiratory antibiotics may overestimate their effect or underestimate the effect of non-respiratory antibiotics.

Finally, a limitation of this mediation approach is the categorical nature of our mediator and outcome, which in part is due to non-collapsibility in logistic regression models and may lead to underestimation of proportions mediated (32). Where the mediator can effectively be intervened upon, and where assumptions of consistency, exchangeability, and positivity are complied with, future studies should consider implementation of causal mediation analysis using a potential outcomes framework to estimate controlled direct and indirect effects (33). Future studies may also benefit from evaluating the confounding role of factors not measured here such as genetic background, epigenetic effects and airway microbiome interactions. Our study design is also at risk of confounding by indication where severe LRTIs requiring antibiotics may be linked to future asthma diagnoses due to shared underlying susceptibilities such as impaired lung function, genetic predisposition to asthma or early immune dysregulation that increase risk of both early-life LRTIs and later asthma. This makes it challenging to seperate the effect of antibiotics from the effect of the infection, and as a result, our model may overestimate the contribution of systemic antibiotics to the development of asthma. To minimize this, following pharmacoepidemiologic practices when confounding by indication cannot be directly measured, we did not include wheezing symptoms in our definition of LRTI, and conducted sensitivity analysis in non-recurrent wheezers at age 1 (34). Our findings remained consistent in these analyses, suggesting the association between antibiotics and asthma is not soley driven by misclassified early asthma/wheezing.

We also recognize that children living in remote areas, or from lower socioeconomic backgrounds experience a disproportionate burden of infection and asthma (35, 36). We address potential confounding of this nature by adjusting all analyses by factors such as study site and family income. Nonetheless, given that our definition of infection severity is based on healthcare utilization, future studies on populations with limited access to care are needed to assess the generalizability of our findings to these groups of children. While our study focuses on the impact of early-life antibiotic use in the general population, it is crucial to recognize that high-risk patient populations or cases of severe infections require distinct clinical considerations, and we do not advocate for withholding antibiotics in cases when they are necessary. Additionally, given the diversity of healthcare systems worldwide, further research in settings outside Canada is needed to assess the generalizability of our findings to populations with differing healthcare practices and needs. Finally, considering that antibiotic prescriptions from our study occurred during the 2010–2012 period, prescribing practices may have since evolved due to initiatives like "Choosing Wisely Canada", potentially leading to fewer infants receiving antibiotics for viral LRTIs. Future studies should attempt to replicate these associations using current datasets to account for changes in clinical practices and to validate these findings in a contemporary cohort.

Overall, our study provides novel evidence that both the severity and management of the infection, rather than simply the occurrence of a LRTI *per se*, may be relevant to asthma development. This highlights the importance of prudent antibiotic use, especially considering the potential long-term effects of these treatments and contribution of unnecessary use to rising antibiotic resistance. While our study illuminates potential links and mechanisms, it also emphasizes the complexity of asthma development and the multifaceted influence of early-life exposures. Ultimately, our results are based on observational data, and the significance of antibiotic use as a mediator in this study does not necessarily confirm causality. There is need for further research involving a finer examination of types of respiratory infection, severity of infection, and nature of antibiotic usage to elucidate these relationships and inform strategies to mitigate risk of developing asthma.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: data from the CHILD Cohort Study is available to researchers upon request. Requests to access these

datasets should be directed to https://childstudy.ca/for-researchers/study-data.

Ethics statement

The studies involving humans were approved by University of British Columbia, University of Alberta, University of Manitoba, The Hospital for Sick Children and McMaster University's respective ethics review boards. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

MM: Conceptualization, Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft. MR: Conceptualization, Methodology, Supervision, Writing – review & editing, Software. DD: Writing – review & editing, Data curation. GW: Data curation, Writing – review & editing. FB: Project administration, Writing – review & editing. RV: Writing – review & editing. EN: Data curation, Writing – review & editing. NR: Data curation, Writing – review & editing. ES: Project administration, Writing – review & editing. PM: Project administration, Writing – review & editing. MA: Funding acquisition, Methodology, Writing – review & editing. ST: Data curation, Project administration, Writing – review & editing. TM: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing, Project administration. PS: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. The Canadian Institutes of Health Research (CIHR), Debbie and Donald H. Morrison Family Foundation and the Allergy, Genes, and Environment Network of Centres of Excellence (AllerGen NCE) provided core support for the CHILD Cohort Study (grants to founding CHILD Study director MS and current director PS). MVM is funded by the CIHR–Institute of Circulatory and Respiratory Health Canada Doctoral Research Award, the CIHR–Canadian Lung Association Research Studentship, and the SickKids Hospital Restracomp PhD Scholarship. MER is funded by a CIHR Canada Graduate Scholarship Doctoral Award. DLYD is funded by a CIHR Frederick Banting and Charles Best Canada Graduate Scholarship Doctoral Award and the University of British Columbia Four Year Doctoral Fellowship. FB is a Simon Fraser University Distinguished Professor. PJM is funded by Women's and Children's Health Research Institute (WCHRI). MBA holds a Tier 2 Canada Research Chair in the Developmental Origins of Chronic Disease and is a Fellow of the CIFAR Humans and the Microbiome Program. SET holds a Tier 1 Canada Research Chair in Pediatric Precision Health and the Aubrey J. Tingle Professor of Pediatric Immunology. PS holds a Tier 1 Canada Research Chair in Pediatric Asthma and Lung Health.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/falgy.2024.1463867/full#supplementary-material

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frontiers | Frontiers in Pediatrics

TYPE Original Research
PUBLISHED 27 February 2025
DOI 10.3389/fped.2025.1439841

OPEN ACCESS

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RECEIVED 28 May 2024
ACCEPTED 17 February 2025
PUBLISHED 27 February 2025

CITATION
Jiang W-y, Jiao R-h, Ma S-l, Dai J-s, Zhu H-f, Wu M-y, Che Y-r, Zhang L and Ding X-y (2025) Serum inflammatory factors, vitamin D levels, and asthma severity in children with comorbid asthma and obesity/overweight: a comparative study.
Front. Pediatr. 13:1439841.
doi: 10.3389/fped.2025.1439841

Serum inflammatory factors, vitamin D levels, and asthma severity in children with comorbid asthma and obesity/overweight: a comparative study

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Objective: To investigate serum inflammatory factors, vitamin D levels, and asthma severity in children with comorbid asthma and obesity/overweight, compared with those with asthma or obesity/overweight alone.
Methods: This retrospective comparative study included children suffering from asthma alone, asthma combined with obesity/overweight, or obesity/overweight alone at Shanghai Pudong New Area People's Hospital between January 2020 and December 2021.
Results: A total of 168 children (mean age: 4.32 ± 1.64 years; 117 males) were included. Compared with children with asthma alone ($n = 56$), those with comorbid asthma and obesity/overweight ($n = 56$) exhibited higher levels of serum levels of interleukin 6 (IL-6) (35.75 ± 24.56 vs. 15.40 ± 19.67), TNF-α (15.44 ± 7.35 vs. 12.16 ± 7.24), and leptin (3.89 ± 3.81 vs. 1.27 ± 2.31), and lower levels of 25-hydroxycholecalciferol (25-(OH) D_3) (26.03 ± 10.77 vs. 37.15 ± 13.35), IL-10 (8.69 ± 2.76 vs. 15.32 ± 6.28), and IL-13 (449.40 ± 315.37 vs. 605.27 ± 351.02) (all $P < 0.05$). Compared with children with obese/overweight alone ($n = 56$), those with comorbid asthma and obesity/overweight had lower IL-10 (8.69 ± 2.76 vs. 12.29 ± 6.61) and higher IL-6 (35.75 ± 24.56 vs. 20.53 ± 17.07), IL-13 (449.40 ± 315.37 vs. 309.47 ± 257.45), and leptin (3.89 ± 3.81 vs. 2.48 ± 3.52) (all $P < 0.05$). Children with comorbid asthma and obesity/overweight showed higher Preschool Respiratory Assessment Measure (PRAM) scores (3.14 ± 2.40 vs. 1.93 ± 1.02, $P = 0.008$) and longer hospital stays (5.96 ± 1.25 vs. 5.29 ± 1.36 days, $P = 0.007$) compared to those with asthma alone.
Conclusions: Significant differences were observed in IL-6, IL-10, IL-13, 25-(OH) D_3 levels, and leptin among children with asthma combined with obesity/overweight and those with asthma or obesity/overweight alone. Children with obesity/overweight alone displayed more severe clinical manifestations and longer hospital stays compared with those with asthma alone.

KEYWORDS

vitamin D, obesity, asthma severity, pediatric asthma, inflammatory factors, retrospective comparative study

Introduction

Pediatric asthma is a chronic inflammatory disease of the airways in children, characterized by airflow obstruction. According to the Global Asthma Report (GAR), 262 million people had asthma in 2019, and 1 in 10 children had asthma symptoms worldwide (1). The global epidemic of asthma in children and adults continues to rise (2). Environmental factors (such as air pollution, pollens, mold, pets, and weather conditions), host factors (such as obesity, nutritional factors, infections, and allergic sensitization), and genetic factors (i.e., asthma susceptibility genes) interact to influence the occurrence and severity of asthma (3).

Childhood obesity is another serious public health issue worldwide, putting children and adolescents at risk of poor health during childhood and adulthood. In China, 6.8% of children <6 years are overweight and 3.6% are obese. In the recent decade, the prevalence of overweight and obesity among Chinese children has been increasing (4). Among children and adolescents aged 6–17 years, 11.1% are overweight and 7.9% are obese (5). Obesity/overweight is a major risk factor for asthma and a complex interaction between obesity and asthma results in higher severity of asthma and poorer control of asthma symptoms (6). In obese individuals, multiple inflammatory mediators and M1 macrophage infiltration are increased in adipose tissues, causing inflammation. The inflammatory process also increases the synthesis of proinflammatory cytokines such as interleukin (IL)-6, tumor necrosis factor-α (TNF-α), IL-1β, and transforming growth factor-β (TGF-β), all contributing to asthma pathogenesis and severity (7). Obesity-related asthma is associated with a Th1 immune response (involving TNF-α, IFN-γ, IL-6, and IL-8) rather than a Th2 response (which involved IL-4, IL-5, IL-10, and IL-13) (8). Nevertheless, the role of inflammatory factors in obese patients with asthma remains unclear.

Vitamin D is an essential nutrient and is required for immune regulation (9, 10). 25-hydroxycholecalciferol (25-(OH) D_3) is a form of vitamin D that the body produces or absorbs from animal sources. The other form of vitamin D is 25-hydroxyvitamin D_2 (ergocalciferol), which comes from plant sources. The total level of 25-(OH)D_3 in the blood is the primary measurement used to assess vitamin D status (11). Obesity and vitamin D deficiency (serum levels <30 ng/ml) have been associated with more severe asthma symptoms (12, 13). The Childhood Asthma Management Program (CAMP) revealed that 35% of children aged 5–12 years had mild to moderate asthma and vitamin D deficiency (<30 ng/ml) (14). Moreover, a study that used the National Health and Nutrition Examination Survey (NHANES) found a significant correlation between vitamin D deficiency [defined as serum 25-(OH) D_3 levels <30 ng/ml] and asthma symptoms in children. Another study associated vitamin D deficiency with poor lung function in obese children (15). Taken together, vitamin D deficiency is seen in obesity and asthma, but the exact interplay among the three in children remains to be defined.

Therefore, this study aimed to investigate serum inflammatory factors, vitamin D levels, and asthma severity in children with comorbid asthma and obesity/overweight, compared with those with asthma or obesity/overweight alone. The results could help refine our understanding of the epidemiology and pathogenesis of asthma in children with obesity/overweight.

Methods

Study design and patients

The retrospective comparative study included children with asthma alone, comorbid asthma and obesity/overweight, or obesity/overweight alone, hospitalized (those with asthma) or attending the outpatient clinic (those with obesity/overweight alone) at Shanghai Pudong New Area People's Hospital between January 2020 and December 2021. This study was approved by the Ethics Committee of Shanghai Pudong New Area People's Hospital (approval #prylz2020-101). The requirement for individual consent was waived by the committee due to the retrospective nature of the study.

The inclusion criteria were (1) children aged 1–11 years and (2) diagnosed with asthma according to the criteria in the "Guideline for the Diagnosis and Optimal Management of Asthma in Children (2016)" (16). The exclusion criterion was wheezing due to other conditions (e.g., congenital heart disease, gastroesophageal reflux, or bronchopulmonary dysplasia).

Data collection

Obese/overweight was defined according to the "Chinese Preschool Children Growth Reference Standard and Related Curve: Based on GAMLSS Approach" (17). A BMI of ≥P_{95} was considered obese, and a BMI of ≥P_{85} was considered overweight (17). The children were categorized into children with asthma alone, obese/overweight alone, and obese/overweight and asthma comorbidity (hereafter referred to as those with comorbid asthma and obesity/overweight). The age, sex, height, and weight of the selected subjects were recorded. Age was accurate to 1 month, height to 1 cm, and weight to 0.1 kg. The PRAM score, length of hospital stay, hospitalization cost, and systemic glucocorticoid use were collected in children with asthma. The discharge criteria included no symptoms of wheezing or shortness of breath and the absence of pulmonary rales. The Preschool Respiratory Assessment Measure (PRAM) was used to evaluate asthma severity (18).

On the day of hospital admission, blood was collected for laboratory testing. Fasting venous blood (3 ml) was collected, held at room temperature for 30 min, and centrifuged at 3,000 r/min for 5 min to collect the serum. Separated serum was stored at −80°C until further use. The 25-(OH) D_3 levels were determined using the electrochemical luminescence method from Roche

(Basel, Switzerland). Leptin (kit no. CSB-E04649h), IL-6 (kit no. CSB-E04638h), TNF-α (kit no. CSB-E04740h), IL-10 (kit no. CSB-E04593h), IL-4 (kit no. CSB-E04633h), and IL-13 (kit no. CSB-E04601h) levels were determined by ELISA, following manufacturer's instructions (Cusabio Technology LLC, Wuhan, China). A Microlab STAR automatic enzyme immunoassay analyzer (Hamilton Co., Reno, NV, USA) was used for measurements.

Sample size

The sample size for this study was calculated to ensure sufficient power to detect significant differences in serum inflammatory factors, vitamin D levels, and asthma severity among the three groups: asthma alone, asthma combined with obesity/overweight, and obesity/overweight alone.

According to preliminary investigation data by the authors (10 cases in each group), the mean levels of IL-6 were 13.45 ± 17.69 pg/ml for asthma, 39.67 ± 21.58 pg/ml for asthma and obesity, and 22.05 ± 22.13 pg/ml for obesity. Using the G*Power 3.1.9.2 software, the effect size (Cohen's d for ANOVA) was calculated as 0.49. Using a two-sided significance level (α) of 0.05 and a power (1-β) of 0.90, it was calculated that a total of 57 patients would be needed. Considering that IL-6 levels do not conform to a normal distribution, the number of patients needed to be increased by 15%. In addition, considering a missing rate of 10%, the minimum sample size required was $n = 57 \times 115\% \times 110\%$, which is approximately equal to 24 patients in each group. Based on the financial and material resources of this study, 56 samples were surveyed in each group to meet the minimum sample size requirement.

Statistical analysis

SPSS 23.0 (IBM, Armonk, NY, USA) was used for statistical analysis. The figures were drawn using the "ggplot2" 3.4.4 package in R 4.3.1. Continuous variables were described as means ± SD. Normally distributed variables were tested using the independent sample t-test (two groups) and one-way ANOVA (multiple groups). For data with a non-normal distribution, the Mann–Whitney *U* test was used to compare two groups, and the Kruskal–Wallis H test was used to compare multiple groups. The Bonferroni method was used to control for false positives. Categorical variables were described as *n* (%) and analyzed using the chi-squared test or Fisher's exact test. Fisher's exact test when the expected number of observations in any of the cells of a contingency table was below 5, or below 10 when there was only one degree of freedom; otherwise, the chi-squared test was used. Therefore, the chi-squared test was used for sex and systemic glucocorticoid use. Spearman correlation analysis was conducted to analyze the correlations among variables. "Systemic glucocorticoid use" is a categorical (binary) variable; after it was encoded as 0/1, the Spearman correlation coefficient with vitamin D was calculated, which is a point-biserial correlation coefficient. Two-sided *P*-values < 0.05 were considered statistically significant.

Results

Characteristics of the children

The eligible patients treated between January 2020 and December 2021 were included. A total of 168 children (117 males; mean age of 4.32 ± 1.64 years) were included. The patients were grouped according to their condition: asthma, obese/overweight, and comorbid asthma and obese/overweight. There were no significant differences in sex or age among the three groups (all $P > 0.05$). Compared with children with asthma alone ($n = 56$), those with comorbid asthma and obesity/overweight ($n = 56$) and obesity/overweight ($n = 56$) had significantly higher BMI (19.70 ± 2.33 and 19.35 ± 2.20 vs. 16.16 ± 0.71, both $P < 0.05$) (Table 1).

Serum cytokine levels

Then, serum levels of proinflammatory and anti-inflammatory cytokines were compared among the three groups to determine whether comorbid asthma and obesity/overweight influenced the cytokine levels compared with asthma alone or obesity/overweight alone. Compared with children with asthma alone ($n = 56$), those with comorbid asthma and obesity/overweight ($n = 56$) exhibited significantly higher levels of serum levels of interleukin 6 (IL-6) (35.75 ± 24.56 vs. 15.40 ± 19.67), TNF-α

TABLE 1 Demographic characteristics.

Variables	Asthma group (*n* = 56)	Comorbidity group (*n* = 56)	Obese/overweight group (*n* = 56)	*P*
Age (y)	4.39 ± 1.57	4.46 ± 1.77	4.13 ± 1.59	0.262
Sex				0.554[a]
Male	40 (71.4%)	36 (64.3%)	41 (73.2%)	
Female	16 (28.6%)	20 (35.7%)	15 (26.8%)	
Body mass index (kg/m^2)	16.16 ± 0.71	19.70 ± 2.33*	19.35 ± 2.20*	<0.001

The *p*-values presented in the final column of the table were unadjusted. The *p*-values denoted with *signified *post-hoc* comparisons that were adjusted through the Bonferroni correction method.

*$P < 0.05$ vs. children with asthma alone.

[a]Chi-squared test.

(15.44 ± 7.35 vs. 12.16 ± 7.24), and leptin (3.89 ± 3.81 vs. 1.27 ± 2.31), and lower levels of 25-(OH) D_3 (26.03 ± 10.77 vs. 37.15 ± 13.35), IL-10 (8.69 ± 2.76 vs. 15.32 ± 6.28), and IL-13 (449.40 ± 315.37 vs. 605.27 ± 351.02) (all $P < 0.05$). Compared with children with obese/overweight alone ($n = 56$), those with comorbid asthma and obesity/overweight had significantly lower IL-10 (8.69 ± 2.76 vs. 12.29 ± 6.61) and higher IL-6 (35.75 ± 24.56 vs. 20.53 ± 17.07), IL-13 (449.40 ± 315.37 vs. 309.47 ± 257.45), and leptin (3.89 ± 3.81 vs. 2.48 ± 3.52) (all $P < 0.05$) (Table 2; Figures 1, 2). These results suggested the combined effect of comorbid asthma and obesity/overweight on the levels of several cytokines.

Asthma symptoms and severity

In order to investigate whether obesity/overweight influenced asthma, the severity of asthma was compared between the asthma alone and comorbid asthma and obesity/overweight groups to examine the impact of obesity/overweight on asthma. Compared with children with asthma alone, those with comorbid asthma and obesity/overweight had significantly higher PRAM scores (3.14 ± 2.40 vs. 1.93 ± 1.02, $P = 0.008$) and longer length of hospital stay (5.96 ± 1.25 vs. 5.29 ± 1.36, $P = 0.007$). There were no significant differences in hospitalization cost and systemic glucocorticoid use between children with asthma alone and comorbid asthma and obesity/overweight (Table 3). Hence, obesity/overweight appeared to exacerbate asthma.

Correlations

Finally, correlations were examined between indicators of obesity and inflammation markers. BMI was negatively correlated with 25-(OH) D_3 ($r = -0.284$, $P < 0.001$), IL-10 ($r = -0.181$, $P = 0.019$), and IL-13 ($r = -0.188$, $P = 0.015$). BMI was positively correlated with IL-6 ($r = 0.386$, $P < 0.001$), TNF-α ($r = 0.172$, $P = 0.026$), and leptin ($r = 0.383$, $P < 0.001$) (Table 4). Vitamin D was negatively correlated with IL-6 ($r = -0.160$, $P = 0.038$) and leptin ($r = -0.155$, $P = 0.045$). Vitamin D was positively correlated with IL-10 ($r = 0.229$, $P = 0.003$) (Table 5).

Discussion

The present study showed that children with comorbid asthma and obesity/overweight had higher IL-6, TNF-α, and leptin, and lower 25-(OH) D_3, IL-10, and IL-13 than children with asthma alone, and had lower IL-10 and higher IL-6, IL-13, and leptin than children with obesity/overweight alone. Compared with children with asthma alone, those with comorbid asthma and obesity/overweight had a higher PRAM score and longer length of hospital stay. These findings highlight the importance of obesity in the management of pediatric asthma.

In the current study, children with obesity/overweight and children with comorbid obesity/overweight and asthma showed higher IL-6 than children with asthma, which illustrates that IL-6 is involved in the pathogenesis of asthma in obese children. High IL-6 secretion by brown fat cells in mice causes the failure of brown fat cells to decompose fat or metabolize glucose and other substances, resulting in obesity and other related complications (19), supporting the present study. In addition, previous studies showed that obese children had a higher Th1 proportion and inflammatory cytokines (IL-6, IFN-γ, and TNF-α) levels (20, 21).

In this present study, the leptin levels in those with comorbid asthma and obesity/overweight were substantially higher than in children with obesity/overweight alone, while leptin expression in children with obesity/overweight alone was higher than in children with asthma alone. The comorbid asthma and overweight/obesity group had a lower expression of IL-10 and IL-13 than children with asthma alone, suggesting that the expression of Th2-related inflammatory factors was higher in children with asthma but not in children with comorbidity. However, the present study did not include healthy controls to verify the mechanism of leptin involvement in asthma. As observed in the literature, obese individuals have higher leptin expression, which in turn stimulates fat cells to secrete proinflammatory mediators such as IL-6, TNF-α, and IL-12 (22, 23). Leptin stimulates the secretion of IL-6 and TNF-α in human peripheral blood mononuclear cells (24). Leptin levels are higher in patients with asthma than in healthy controls, and leptin expression is significantly higher in patients with worsening asthma symptoms than in asymptomatic patients (25). Taken

TABLE 2 Comparison of proinflammatory mediators and 25-(OH) D3 in the three groups of children.

Indicators	Asthma group ($n = 56$)	Comorbidity group ($n = 56$)	Obese/overweight group ($n = 56$)	P
25-(OH) D_3, ng/ml	37.15 ± 13.35	26.03 ± 10.77*	27.45 ± 11.27*	<0.001
IL-6, pg/ml	15.40 ± 19.67	35.75 ± 24.56*	20.53 ± 17.07*,#	<0.001
TNF-α, pg/ml	12.16 ± 7.24	15.44 ± 7.35*	13.77 ± 8.03	0.013
IL-10, pg/ml	15.32 ± 6.28	8.69 ± 2.76*	12.29 ± 6.61*,#	<0.001
IL-13, pg/ml	605.27 ± 351.02	449.40 ± 315.37*	309.47 ± 257.45*,#	<0.001
Leptin, ng/ml	1.27 ± 2.31	3.89 ± 3.81*	2.48 ± 3.52*,#	<0.001
IL-4, pg/ml	47.80 ± 36.15	56.90 ± 44.83	50.33 ± 40.93	0.741

25-(OH) D_3, 25-hydroxycholecalciferol; IL-6, interleukin 6; TNF-α, tumor necrosis factor-α; IL-10, interleukin 10; IL-13, interleukin 13; IL-4, interleukin 4.

The *p*-values presented in the final column of the table were unadjusted. The *p*-values denoted with * and # signified *post-hoc* comparisons that were adjusted through the Bonferroni correction method.

The data were non-normally distributed. The global *P*-values were from the Kruskal–Wallis test. The Mann–Whitney test was used for the *post hoc* testing of pairs of groups: *$P < 0.05$ vs. the asthma group; #$P < 0.05$ vs. the comorbidity group.

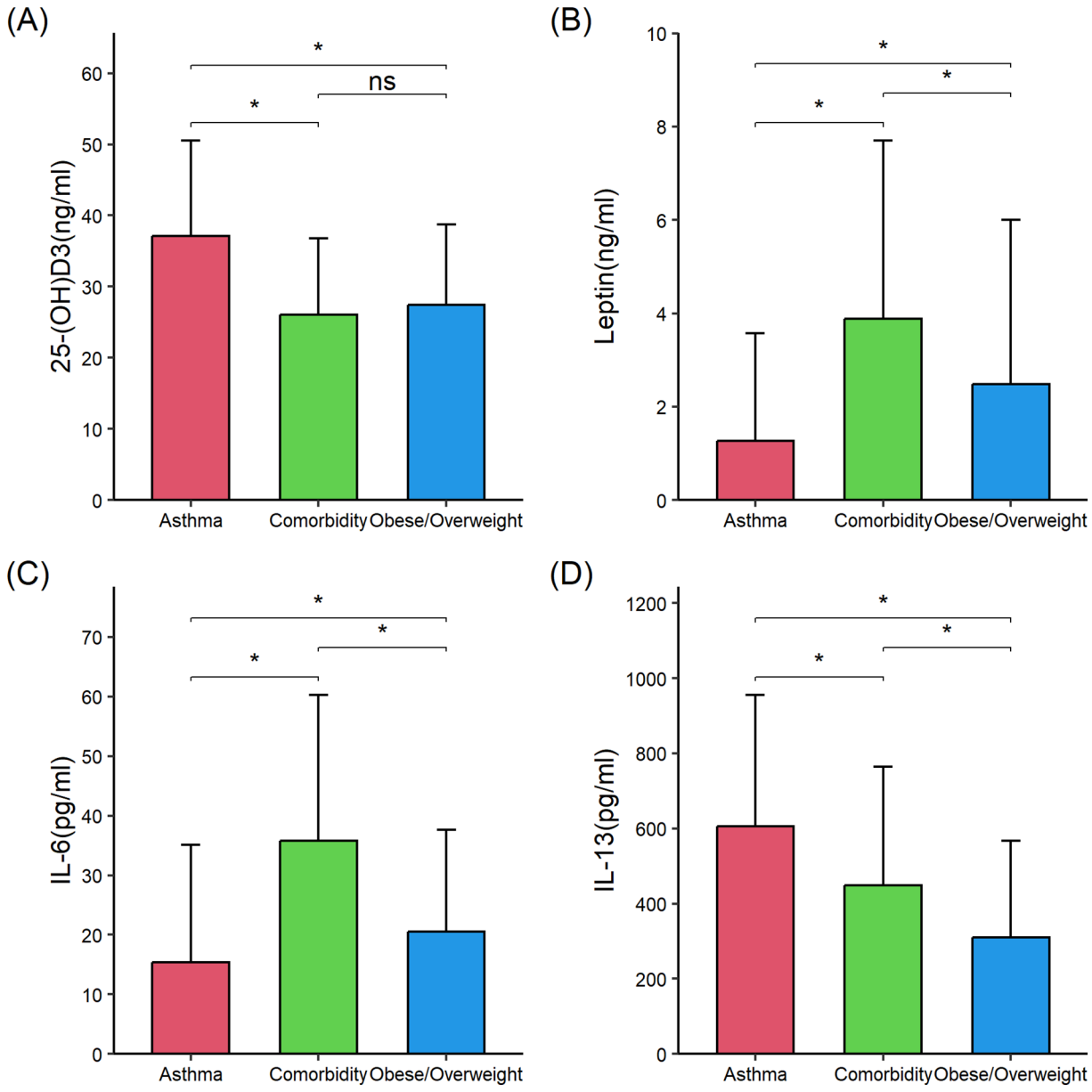

FIGURE 1
Comparison of 25-(OH) D3, leptin, interleukin (IL)-6, and IL-13 in the three groups of children. **(A)** 25-(OH)D_3. **(B)** Leptin. **(C)** IL-6. **(D)** IL-13. 25-(OH) D_3, 25-hydroxycholecalciferol; IL-6, interleukin 6; IL-13, interleukin 13. The data were non-normally distributed. The global *P*-values were from the Kruskal–Wallis test. The Mann–Whitney test was used for the *post hoc* testing of pairs of groups. Statistical significance between groups is indicated as follows: $*P < 0.05$; ns (not significant) $P \geq 0.05$. The figure was drawn using the "ggplot2" 3.4.4 package in R 4.3.1. Error bars represented standard deviation.

together, these findings suggest that leptin is involved in the pathogenesis of obesity-related asthma. Leptin promotes the differentiation and activation of Th1 cells, inhibits the production of the Th2 cytokines (such as IL-4, IL-5, and IL-10), and activates the proinflammatory Th17 cytokines (26–28).

In the present study, BMI was moderately and positively correlated with leptin and IL-6. A previous study found that the obesity-related proinflammatory cytokine IL-6 was related to asthma severity when metabolic syndrome co-occurred (29). Vitamin D reduces inflammation through various mechanisms, including inhibition of NF-κB signaling, P38 MAP kinase phosphorylation, and activating macrophages, B cells, T cells, neutrophils, dendritic cells, and mast cells (30, 31). Moreover, vitamin D deficiency was found to be associated with obesity and asthma (14). Vitamin D increases the expression of CD14 in lung epithelial cells and macrophages and participates in local defense mechanisms (32). Vitamin D regulates the expression of bronchial vascular endothelial growth factor, fibronectin, and IL-6 in bronchial smooth muscle, thereby reducing airway inflammation (33–35). Children with asthma and obesity have

10.3389/fped.2025.1439841

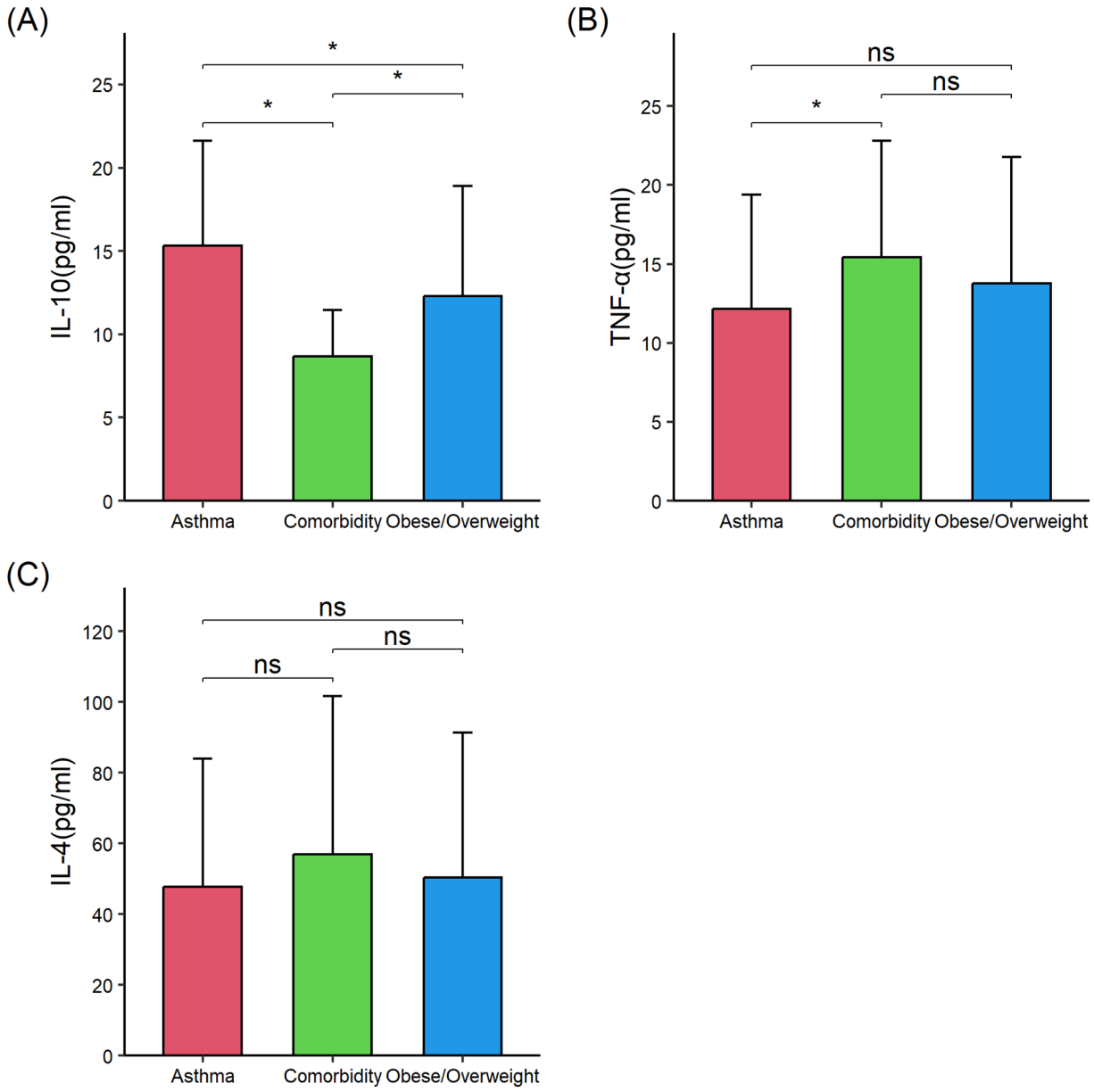

FIGURE 2
Comparison of interleukin (IL)-10, tumor necrosis factor (TNF)-α, and IL-4 in the three groups of children. **(A)** IL-10. **(B)** TNF-α. **(C)** IL-4. IL-10, interleukin 10; TNF-α, tumor necrosis factor-α; IL-4, interleukin 4. The data were non-normally distributed. The global *P*-values were from the Kruskal–Wallis test. The Mann–Whitney test was used for the *post hoc* testing of pairs of groups. Statistical significance between groups is indicated as follows: * $P < 0.05$; ns (not significant) $P \geq 0.05$. The figure was drawn using the "ggplot2" 3.4.4 package in R 4.3.1. Error bars represented standard deviation.

been reported to have lower serum vitamin D levels (15, 36). In a previous study, 29% of overweight children (BMI: 85th–95th percentile for age and gender), 34% of obese children (BMI: 95th–99th percentile), and 49% of severely obese children (BMI > 99th percentile) had 25(OH) D_3 levels of <30 ng/ml (36). Serum vitamin D levels are inversely correlated with body fat levels, partly due to increased vitamin D storage in adipose tissue (37). Vitamin D deficiency is associated with the acute exacerbation of asthma and glucocorticoid resistance (38, 39). Vitamin D increases the bioavailability of glucocorticoids in airway smooth muscle cells and exerts a protective effect on Th1/Th2-driven airway inflammation (40). Nevertheless, the interaction between asthma, obesity, and vitamin D deficiency is complex.

In the present study, BMI was negatively correlated with 25-(OH) D_3, while the levels of 25-(OH) D_3 in children with comorbid asthma and obesity/overweight and children with obese/overweight alone were lower than children with asthma alone and the normal standard (<30 ng/ml). Nevertheless, there were no significant differences between those with comorbid

TABLE 3 Comparison of asthma severity and other parameters between children with asthma alone and those with comorbid asthma and obesity/overweight.

Parameters	Asthma group (n = 56)	Comorbidity group (n = 56)	P
PRAM score	1.93 ± 1.02	3.14 ± 2.40	0.008[a]
Length of hospital stay (d)	5.29 ± 1.36	5.96 ± 1.25	0.007[a]
Hospitalization cost (CNY)	3,593.79 ± 772.62	3,656.74 ± 720.22	0.364[a]
Systemic Glucocorticoid use	27 (48.2%)	35 (62.5%)	0.128[b]

PRAM, the preschool respiratory assessment measure.
[a]Mann–Whitney test.
[b]Chi-squared test.

TABLE 4 Correlation of BMI with vitamin D and proinflammatory mediators.

	Correlation coefficient (r)	P
25-(OH) D_3	−0.284	<0.001
IL-6	0.386	<0.001
TNF-α	0.172	0.026
IL-10	−0.181	0.019
IL-13	−0.188	0.015
Leptin	0.383	<0.001

25-(OH) D_3, 25-hydroxycholecalciferol; IL-6, interleukin 6; TNF-α, tumor necrosis factor-α; IL-10, interleukin 10; IL-13, interleukin 13.

TABLE 5 Correlation of vitamin D with pro inflammatory mediators and clinical characteristics.

	Correlation coefficient (r)	P
IL-6	−0.160	0.038
TNF-α	−0.012	0.875
IL-10	0.229	0.003
IL-13	0.126	0.104
Leptin	−0.155	0.045
PRAM score	0.089	0.353
Length of hospital stay (d)	0.076	0.424
Hospitalization cost (CNY)	−0.091	0.343

IL-6, interleukin 6; TNF-α, tumor necrosis factor-α; IL-10, interleukin 10; IL-13, interleukin 13.

asthma and obesity/overweight and children with obesity/overweight alone. The present study examined the correlation between vitamin D and various proinflammatory factors, and vitamin D had a weak negative correlation with IL-6 and leptin, as well as a weak positive correlation with IL-10. Krajewska et al. found that vitamin D intake seemed to exert its anti-inflammatory effect mainly via decreasing the CRP level and protecting stable values of IL-10 (41). It suggests that 25-(OH)D_3 may be involved in the regulation of relevant inflammatory factors. Still, vitamin D levels were not associated with the severity or length of hospital stay in children with asthma. Overall, vitamin D deficiency hinders the immune regulation in obese children.

According to the PRAM scores, the children with comorbidity suffered from more severe asthma symptoms, including difficulty breathing, compared with children with asthma alone. Nevertheless, systemic glucocorticoid administration did not produce any significant difference in asthma severity between the two groups. Children with comorbidity stayed longer in the hospital and suffered from more severe asthma symptoms with no ease with glucocorticoid administration, implying that obesity-related asthma may have had glucocorticoid resistance. Obese children displayed more severe clinical manifestations, and asthma control was very difficult in those children (42). The interaction between obesity and pulmonary disorders is multifaceted. Obesity alters chest wall dynamics, directly affecting the thorax biomechanics. In a previous study, obesity was positively associated with the length of hospital stay and the need for mechanical ventilation in children with asthma (43).

The present study has some limitations. Due to the absence of a normal control group in this study, it was impossible to compare the inflammatory factors of the disease groups with children without asthma or obesity/overweight. Since the data were retrieved from a single center, generalizability is limited. The study did not investigate the physical activity of the included children, family economy, or culture. Since it was a retrospective study, the families could not be contacted to collect such data. This study did not reexamine the inflammatory factors during the stable phase of asthma; therefore, the changes in the expression of these inflammatory factors after the treatment could not be speculated. Further prospective studies are necessary to investigate whether vitamin D supplementation or effective control of body weight can normalize the inflammatory factors and ease the severity of asthma in children with obesity-associated asthma.

In conclusion, there were significant differences in IL-6, IL-10, IL-13, 25-(OH) D_3 levels, and leptin levels among children with asthma combined with obesity/overweight and those with asthma or obesity/overweight alone. Furthermore, those with obesity/overweight and asthma may display more severe clinical manifestations and longer hospital stays compared to children with asthma alone.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Ethic Committee of Shanghai Pudong New Area People's Hospital (No. prylz2020-101). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

W-yJ: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. R-hJ: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. S-lM: Data curation, Investigation, Methodology, Writing – review & editing. J-sD: Data curation, Investigation, Methodology, Writing – review & editing. H-fZ: Data curation, Investigation, Methodology, Writing – review & editing. M-yW: Data curation, Investigation, Methodology, Writing – review & editing. Y-rC: Data curation, Investigation, Methodology, Writing – review & editing. LZ: Conceptualization, Formal analysis, Writing – review & editing. X-yD: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by the Outstanding Young Medical Talent Training Program of the Pudong Health Bureau of Shanghai funding (No. PWRq2020-38) and the Youth Project of Shanghai Municipal Health Commission Scientific Research funding (No. 20204Y0110).

Acknowledgments

The authors thank the children and their parents for participating in this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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frontiers | Frontiers in Allergy

TYPE Mini Review
PUBLISHED 30 April 2025
DOI 10.3389/falgy.2025.1584928

OPEN ACCESS

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RECEIVED 28 February 2025
ACCEPTED 18 April 2025
PUBLISHED 30 April 2025

CITATION
Adamko DJ and Hildebrand KJ (2025) The changing epidemiology of paediatric childhood asthma and allergy in different regions of the world.
Front. Allergy 6:1584928.
doi: 10.3389/falgy.2025.1584928

The changing epidemiology of paediatric childhood asthma and allergy in different regions of the world

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Allergic disorders encompass a variety of conditions including asthma, atopic dermatitis, food allergy, allergic rhinitis, and eosinophilic esophagitis. These atopic disorders are connected via an abnormal host immune response to the environment. A series of longitudinal cross-sectional studies conducted over the past 3 decades have reported on the epidemiological trends that contribute towards the development of pediatric asthma and allergic disease. Infant birth cohort studies assessing the microbiome have offered clues as to the underlying biological mechanisms and basis for allergic disease. Why this abnormal immune response is occurring is the basis of decades of research and the reasons for this chapter. Our understanding of the biology of the immune system has increased exponentially with the advances in genomic testing, providing further opportunity for targeted treatments and more importantly, primary prevention of atopic disease.

KEYWORDS

asthma, allergy, epidemiology, inborn error immunity, primary atopic disorders

Introduction

Asthma and allergic diseases are the most common chronic pediatric conditions and are leading healthcare costs. In 2013, direct costs of pediatric asthma were US$5.92 billion, with the average annual costs per child ranging from US$3,076 to US$13,612 (1). Allergic disorders encompass a variety of conditions including asthma, atopic dermatitis, food allergy, allergic rhinitis, and eosinophilic esophagitis. Allergic disease develops in a time-based sequence, often referred to as the 'atopic march': beginning with atopic dermatitis and food allergy in infancy, to the development of asthma and allergic rhinitis in later childhood or beyond (2, 3). Severe forms of atopic diseases are a manifestation of immune dysregulation and reflect a new category of inborn errors of immunity collectively defined as primary atopic disorders (4). These allergic conditions are connected via an abnormal host immune response to the environment. For reasons that are currently unknown, exposure to common environmental factors like animal dander, certain foods, inhaled molds or pollens are not tolerated but instead generate a T Helper type 2 (T_H2)-like immune response driven by proinflammatory cytokines such as interleukin (IL)-4, IL-5 and IL-13 which activate effectors cells such as mast cells and eosinophils, thus driving inflammation. Classically, the diagnosis of allergic disease is established clinically based on the presence of effector cells like eosinophils or

immunoglobulin E (IgE)-mediated activation of mast cells and/or basophils. These immune cells promote an allergic response leading to symptoms of asthma such as paroxysmal cough, dyspnea, and wheezing; for allergic rhinoconjunctivitis symptoms of rhinorrhea, angioedema and conjunctivitis, and urticaria, flushing and possibly hypotension in the setting of anaphylaxis (5).

Understanding the biology driving inflammation is a key goal for research and prevention

Environmental exposures vary in an urban vs. rural setting, which seems to factor into the development of allergic disease. The term 'hygiene hypothesis' was coined in 1989 by Strachnan reflecting an observational theory that children with fewer viral infections in early life were more likely to develop allergic rhinitis (6). Stiemsma et al. summarized the evolution of the immune dysregulation associated with the hygiene hypothesis including the early report of low incidence of rheumatoid arthritis in Western Nigeria where malaria exposure is frequent (7) and how experimental theories have expanded the hygiene hypothesis to also include commensal and symbiotic intestinal microbes (8). One of the earliest studies assessing the role of farm exposures was described by Martinez and Holt by comparing two cross-sectional studies in Europe and concluded that children who lived on farms are exposed to a more diverse range of microbial agents and develop less allergic disease as demonstrated by reduced prevalence of asthma and atopy (9). Stein et al. demonstrated that even among genetically similar populations such as the Amish and Hutterite communities in the United States, environmental exposures as it relates to differing farming practices elicits varying innate immune responses. Amish farmers use a traditional practice with exposure to environments rich in microbes, whereas Hutterites apply industrialised technology. None of the Amish children studied had asthma in contrast to 20% of Hutterite children (10). However, the impact of rural exposures on asthma is complex and in some cases under-diagnosis of asthma may be occurring in these rural/remote communities (11). New research assessing the modality or route of exposure (inhaled, ingested) and effect on the varying tissue microbiomes (respiratory, intestinal, cutaneous) are emerging as a key question in our understanding of causal mechanisms (12). Early life exposures to inflammation generate innate immune responses that vary within and across populations (13). The discovery of T regulatory cells and innate lymphoid cells add to our understanding of the complexities of the immune system in atopic disease development beyond Th1 vs. Th2 imbalances (14). The increased prevalence of allergic disease has been attributed in part to epigenetic mechanisms such as DNA methylation, whereby environmental effects create biochemical changes of transcriptionally relevant genetic information without altering the nucleotide sequence of the genome (15). Evolutionary life history theory considers the relative cost and effectiveness of different immune responses: innate immune responses are lower cost up front however are imperative for novel infections; acquired immune responses are most effective against secondary infections due to antigen-specific antibodies however require nutritional abundance (16). Socioeconomic factors, including access to healthcare, complicate the relationship of location and allergy.

Nutrition also plays a role in homeostasis between the balance of Th_1 and Th_2 immune responses. Micronutrient deficiencies may contribute to atopic diseases: iron, zinc, vitamins A and D deficiencies are drivers of atopic diseases. Children with allergic diseases in the US and China are up to 8 times more likely to be anemic compared to children without allergic disease. Dietary restrictions due to food allergy can also lead to micronutrient deficiencies (17). Gut microbiome studies of semi-nomadic gatherer/hunter people of the Yanomami has been found to be distinctly more diverse when compared to westernized counterparts. Indigenous diets contain high-fiber plant products rich in inulin, which has been shown to stabilize gut homeostasis via short chain fatty acids that inhibit pro-inflammatory cytokines when absorbed by gut epithelial cells (18).

Epidemiological trends of asthma and atopic disease among children and youth

Asthma is one of the most common chronic respiratory conditions of childhood worldwide, but regional differences exist. Several large population-based and cohort studies have shaped our understanding of asthma and allergic disease worldwide, providing early clues into the factors impacting the epidemiology of allergic disease and asthma worldwide including climate change, pollution, nutrition, birthing patterns, use of antibiotics early in life, and environmental exposures including viral infections.

In North America, data from the Center for Disease Control in the United States in 2019–2021 reported the prevalence of asthma among children less than 18 years was 6.5%. Prevalence was higher among youth 15–17 years (9.5%), in Black/African American children (11.6%), in Puerto Rican children (17%), and in those with the lowest household incomes (10.2%). Data from the National Institute of Health reported the prevalence of childhood asthma was 8.4% in 2017 declining to 5.8% in 2020, however the authors caution that a newer data collection strategy, coupled with the COVID19 pandemic reducing the number of viral respiratory tract infections, may be contributing factors to the declining prevalence (19).

The International Study of Asthma and Allergies in Childhood (ISAAC) is the largest study examining the prevalence and severity of asthma symptoms, allergic rhinitis and eczema among children in different parts of the world. The ISAAC studies advanced our understanding of the epidemiology of pediatric asthma, and led to the exploration of environmental factors that might contribute to the asthma and the atopic march at a population level. This cross-sectional study consisted of three phases. ISAAC Phase I (1994–1995) involved the assessment of prevalence and severity of asthma and allergic disease and included 700,000 school-age children from 56 countries using simple core questionnaires that

were easy to implement regardless of health care resources. Phase II investigated possible etiological factors as suggested by Phase I; and Phase III (2011–2003) was a repetition of Phase I to assess trends in prevalence over time. There were two age groups of children studied: 6–7- and 13–14-year-olds. The Phase I study found significant differences in the prevalence of asthma between countries: In the 13–14-year-old age group, the prevalence of wheeze in the preceding 12 months ranged from 2.1 in Indonesia to 31.2% in the United Kingdom (UK) and 4.1 in Indonesia to 32.1% in Costa Rica in the younger age group. Prevalence was higher in countries that were English speaking or Latin America. This study had three countries with 14–15 study sites each, however the prevalence of asthma varied from low in India, moderate in Italy and high in the UK, suggesting discrepancies were most consistent between countries compared to within countries (20).

ISAAC also reported prevalence results for allergic rhinitis and atopic dermatitis. Allergic rhinitis symptom prevalence was reported between 0.8% and 14.9% in the 6–7-year-olds and from 1.4% to 39.7% in the 13–14-year-olds. This variation in reporting of allergic rhinoconjunctivitis symptoms highlighted differences in how allergic disease symptoms are labelled and reported at a population level (21). For atopic eczema, the ISAAC study reported that the highest prevalence rates were seen in countries where asthma rates were not similarly elevated including Scandinavia and African countries (22). The discordance in prevalence for atopic diseases within a country reflects a need for further research as to why some countries seemingly follow an 'atopic march' and others may not.

ISAAC Phase III study included approximately 1,200,000 children from 233 centers in 98 countries. Results found that the global prevalence of current asthma, rhinoconjunctivitis and eczema in the 13–14-year age group was 14.1%, 14.6% and 7.3%, respectively. Consistent with results from the Phase I study, The Phase III study demonstrated a wide variability in the prevalence and severity of asthma, rhinoconjunctivitis and eczema depending on the regions. Additionally, the Phase III study further established that the prevalence of allergic disease is high in non-affluent centres with low socioeconomic conditions. This suggests local environment characteristics play a key role in determining the differences in prevalence between one place and another (23).

To improve our understanding of asthma symptoms in younger children, the International Study of Wheezing Infants (EISL) was performed as a multicenter, cross-sectional study assessing the prevalence of recurrent wheezing and related risk factors in infants during the first year of life. This study revised the ISAAC questionnaire and validated it for infants less than 12 months of age and administered the survey between 12 and 15 m of age. In 2005, 30,093 children participated from 17 centers [25,030 in Latin American (LA) countries in 12 sites; and 5063 from 5 European (EU) sites]. Of the results reported, symptoms of a viral infection in the first 3 months of life were the most consistent factor associated with a shorter time to first episode of wheeze (24).

More recently the Global Asthma Network (GAN) Phase 1 study published results from a cross-sectional study striving to report on global surveillance of asthma prevalence, severity, management and risk factors. This was the first study since ISAAC that aimed to assess global asthma prevalence over a 27-year period among school age children. Results include a decrease in prevalence for severe asthma symptoms in adolescents however there was an overall increased report of ever having asthma symptoms and night cough in both adolescents and school age children. Key findings of the GAN study indicates that the overall prevalence of asthma remains stable, however 1 in 20 children worldwide has severe asthma (25).

Lastly, the Environmental Influences on Child Health Outcomes (ECHO) study assessed 5,809 children from 10 of the 12 cohorts from the Children's Respiratory and Environmental Workgroup (CREW) birth cohort consortium. This study assigned census-derived tract-level data for socioeconomic, demographic, and housing variables. Study findings included Black and Hispanic children had higher rates of asthma and asthma morbidity compared to White children; Black and Hispanic children in this study resided in communities with economic deprivation at a disproportionate rate compared to White children. Applying census data to the CREW cohorts, the authors reported environmental characteristics at birth relating to population density and poverty were associated with an elevated risk of asthma incidence. Black and Hispanic children were at higher risk than White children for developing asthma regardless of neighbourhood, further linking race, ethnicity and neighbourhood factors with onset of asthma (26).

The role of viruses in the development of asthma

Viruses are important factors in the environment which the host must respond to. This response can be important in the development of asthma and the diagnosis of asthma (27). Numerous studies have shown that infants (<2-year-olds) with a more severe response [i.e., hospitalized with Respiratory Syncytial Virus (RSV) bronchiolitis] are at increased risk of developing later asthma and in some cases even allergic disease (28–32). In addition, there are data that other viruses which cause hospitalization at this young age (i.e., human metapneumovirus) can also increase the risk of later asthma and allergic disease (33). It is also well described that common cold viruses like Rhinovirus are a key trigger for an asthma exacerbation in all ages. People that present to urgent care with Rhinovirus are much more likely to have a diagnosis of asthma (34). The host response to these common viruses is critical to our understanding of allergic disease and asthma (35). For example, there is excellent data *in vivo* and *in vitro* that typically allergic effector cells like eosinophils can be triggered by viral infection to release mediators and induce airway hyperresponsiveness (36, 37). RSV has unique characteristics that could attract allergic effector cells to the airway (38). Overall, the reason why people with allergic inflammation respond so poorly to common colds is an important factor in our understanding of allergic airway disorders.

The role of the microbiome: expanding understanding of genes and environment on development of allergic disease

The Canadian Healthy Infant Longitudinal Development (CHILD) Study is a longitudinal birth cohort study that aims to advance knowledge about the genetic and environmental determinants of atopic diseases including asthma, allergy, allergic rhinitis and eczema. This study recruited 3,500 pregnant women who gave birth between 2009 and 2012 from 4 provinces and followed the infants prospectively. One of the first published reports from this cohort study reported on the gut microbiome of 24 healthy infants and correlated with mode of delivery and feeding status (breastfed or formula fed). Infants born by elective caesarean section had low bacterial diversity and richness. This study advanced understanding of infant gut microbiota and offered new evidence correlating to mode of delivery (39). Another key finding from the CHILD study is the impact of the timing of systemic antibiotics administered within the first year of life are associated with risk of atopic dermatitis [adjusted odds ratio (aOR) = 1.81; 95% CI: 1.28–2.57; $P < 0.001$]. Additionally, antibiotics in the first year of life were linked to infant gut microbiome disruption and elevated atopic dermatitis risk. This study also identified key gut microbiome components that could potentially be used to predict and possibly prevent the onset of atopic dermatitis (40). Antibiotic stewardship programs have successfully reduced the use of antibiotic prescriptions in infants and children: a systematic review of 113 studies assessing antimicrobial stewardship programs in children 0–18 years, 79.6% of the studies showed a significant reduction in inappropriate antibiotic prescriptions (41). A Canadian population level study coupled with a prospective cohort analysis found that declining rates of asthma diagnosis at age 1–4 years was associated with decreased antibiotic use in infancy (age < 1 year). Asthma incidence increased by 24% with each 10% increase in antibiotic prescribing (adjusted incidence rate ratio 1·24 [95% CI: 1·20–1·28]; $p < 0.0001$) (42). Birth cohorts from around the world continue to collect key information and data, providing clues on mechanisms of disease for the development of asthma and atopic disease, and offer strategies towards primary prevention at an individual and population level.

Severe atopy: the tip of the iceberg for uncovering inborn errors of immunity (IEI)

While the environment is important, family history and a genetic basis for disease is well-described. Inborn errors of immunity are a group of disorders caused by damaging germline variants in single genes. The International Union of Immunological Societies (IUIS) has curated and maintained an updated list of inborn errors of immunity since the 1970s. In the United States the incidence of IEIs is 6 per 10,000 individuals (43). Although once considered a rare group of diseases, more recently our understanding has shifted to recognize that collectively these disorders are more common and important for allergic diseases. In the 2024 summary, the IUIS listed 555 IEI's and 17 phenocopies due to genetic variants in 504 different genes (44). Severe atopy including atopic dermatitis, allergic asthma and food allergy are a well described presentation for a group of IEIs categorized as primary atopic disorders (PADs), a term first coined in 2018 to delineate this distinct group. PADs are a group of heritable monogenic allergic disorders, often characterized by severe, early-onset, and co-existent allergic conditions such as atopic dermatitis, food allergy and allergic asthma (45). One of the challenges of PADs is that environmental and host factors can contribute to the heterogeneity of the clinical presentation and severity of disease, requiring clinicians to have a high index of suspicion for IEI, particularly in the absence of infections (46). Our understanding of the biology of these conditions has laid the groundwork for therapies for some of the most common allergic disorders affecting large portions of the population.

Conclusion

Allergic disorders affect a significant proportion of the global population. Abnormal host immune responses towards common antigens in the environment, coupled with genetic predisposition, form the matrix of atopic disease. Epidemiologic data arising from birth cohorts and cross-sectional studies around the world have significantly altered our understanding of the gene-environment interaction as it relates to allergic conditions and factors influencing the risk of developing disease. Our understanding of the biologic and immunologic mechanisms underpinning monogenic forms of the primary atopic disorders characterized by immune dysregulation has contributed towards targeted therapies. Ongoing research and applications of genomic testing to further characterize biological pathways may help pave the transition towards precision medicine for atopic disorders.

Author contributions

DA: Writing – original draft, Writing – review & editing. KJH: Writing – review & editing, Writing – original draft.

Funding

The author(s) declare that no financial support was received for the research and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

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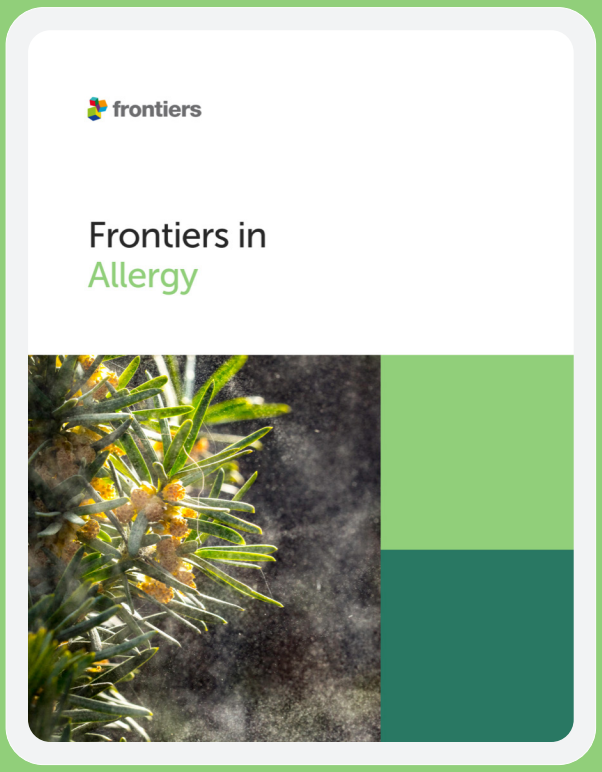

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