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Association of infant antibiotic exposure and risk of childhood asthma: A meta-analysis

Zeyi Zhang, MD, Jingjing Wang, MD, Haixia Wang, MD, Yizhang Li, MD, Yuanmin Jia, MD, Mo Yi, MD and Ou Chen, PhD*

ABSTRACT

Background: Infant antibiotic exposure may be associated with childhood asthma development.

Objective: To examine and detail this association considering potential confounders.

Study design: PubMed, EMBASE, Web of Science, and the Cochrane Library were searched for publications from January 2011 to March 2021. Eligible studies were independently reviewed to extract data and assess quality. Random effect model was used to pool odds ratio (OR) and corresponding 95% confidence intervals (CIs).

Results: A total of 52 studies were included. The association of infant antibiotic exposure and childhood asthma was statistically significant for overall analysis (OR, 1.37; 95% CI, 1.29–1.45) and for studies that addressed reverse causation (RC) and confounding by indication (CbI) (1.19; 95% CI, 1.11–1.28). Significance remained after stratification by adjustment for maternal antibiotic exposure, medical consultation, sex, smoke exposure, parental allergy, birth weight, and delivery mode. In detailed analyses, macrolides (OR, 1.56; 95% CI, 1.31–1.86), antibiotic course≥5 (OR, 1.79; 95% CI, 1.36–2.36), exposure within 1 week of birth (OR, 1.82; 95% CI, 1.34–2.47), asthma developed among 1–3 years (OR, 1.84; 95% CI, 1.63–2.08), short time lag between exposure and asthma onset (OR, 2.05; 95% CI, 1.91–2.20), persistent asthma (OR, 2.61; 95% CI, 1.49–4.59), and atopic asthma (OR, 2.14; 95% CI, 1.58–2.90) showed higher pooled estimates.

Conclusion: Infant antibiotic exposure is associated with increased risk of childhood asthma considering confounding, and the association varied with different settings of exposure and outcomes. This highlights the need for prevention of asthma after early antibiotic exposure. Heterogeneity among studies called for caution when interpretation.

Keywords: Antibiotic, Childhood, Asthma, Confounding, meta-analysis

INTRODUCTION

Asthma, with an estimated worldwide prevalence of 14% among children,[1] is one of the most common children's chronic respiratory diseases. Asthma development is considered to be susceptible to genetic and environmental factors.[2] Human microbiota, as an environmental factor, play a key role in immune modulation, and are thus protective for asthma development.[3-5] Recently,

School of Nursing and Rehabilitation, Cheeloo College of Medicine, Shandong University, #44 West Wenhua Road, Jinan, 250012, China
*Corresponding author. E-mail: chenou@sdu.edu.cn
Full list of author information is available at the end of the article

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numerous studies suggested that infant antibiotic exposure can reduce the gastrointestinal microbiome diversity[6] and affect the balance between microbiota and immune system during a period when microbiome changes rapidly.[7] As a result, antibiotic use in infancy may be particularly considerable to childhood asthma development.

However, studies investigating the association between infant antibiotic exposure and childhood asthma development produced conflicting conclusions. This is because the association is subject to various confounding factors.[8] Besides family, delivery, and environmental factors, remarkably, reverse causation (RC) and confounding by indication (CbI) are 2 strong confounders.[9,10] RC occurs when early-onset symptoms of asthma are treated with antibiotics. CbI occurs when antibiotics are prescribed for respiratory infection (RI), which alters the microbiome through a cascade of host immune responses,[11] and is an independent risk factor for asthma. Both of these factors will lead to false causal effect. Previous meta-analyses published in 2011 pooled studies that addressed RC and CbI showed small effect of antibiotic on childhood asthma.[12,13] However, they included studies with either relatively short follow-up or short exposure period or long interval between exposure and outcome, which may lead to an underestimated effect. Additionally, these reviews lack sufficient details such as antibiotic timing, age of asthma.

Growing number of studies that addressed RC, CbI, and some emerging confounders, as well as details on different settings of antibiotic exposure and asthma outcome have been published since then, with inconsistent findings. Given the conflicting evidence, limitations of the previous meta-analyses, and availability of new data, we conducted the present meta-analysis to examine the association of infant antibiotic exposure and risk of childhood asthma considering the effect of potential confounders, and to detail the association based on different settings of exposure and outcome.

METHOD

This meta-analysis and systematic review was conducted and reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA).[14]

Search strategy

PubMed, EMBASE, Web of Science, the Cochrane Library, and Medline were searched for English publications from January 2011 to March 2021 as there were meta-analyses published in 2011. The following search terms were used: (antibiotic*) and (child OR pediatric OR "early life" OR infan* OR neonatal) and (asthma OR wheezing OR wheeze). Additional studies were found by searching reference lists of relevant articles.

Study selection

Retrieved articles were imported to Endnote. After removing duplications, 2 authors independently reviewed the articles and determined appropriateness for the inclusion. Where consensus on eligibility could not be achieved, a third author was involved. The inclusion criteria were: 1) Studies examined the association of infant antibiotic exposure and childhood asthma or wheezing; 2) Studies were observational studies; 3) Studies provided risk estimates (odds ratio [OR], risk ratio [RR], hazard ratio [HR]) and 95% confidence intervals (CIs); 4) Antibiotic exposure occurred within 3 years of life; and 5) Age of asthma or wheezing ranged from 1 to 18 years. The exclusion criteria were: 1) Studies only examined the effect of antibiotic on asthma exacerbation; 2) Estimate of mixed exposure or outcome was given, rather than a specific estimate of antibiotic exposure or asthma outcome; and 3) Prenatal and postnatal antibiotic exposures were mixed for analysis. Asthma was defined as persistent or recurrent broncho-obstructive symptoms with a good response to bronchodilators and assessed either by questionnaires or doctor's diagnosis or anti-asthmatic drug prescriptions. Studies reporting wheezing as the outcome were also included in the main analysis. Nevertheless, given the fact that not all wheezes are truly a condition of asthma, we conducted detailed analyses stratified by asthma definition and age of "asthma" onset.

Data extraction

Two authors independently extracted data from all eligible studies using a standardized data collection form. The extracted data included: first

author, publication year, country/region, race, study design, sample size, number of cases, antibiotic exposure timing, age of asthma, diagnosis and measurement of exposure and outcome, effect size and corresponding 95% CI, correction for RC and CbI, and adjustment model. If an article contained separate studies, each study was deemed as a single record and its effect size was extracted. If more than 1 article reported the findings of the same study, the effect size of study reporting longer follow-up was extracted. If there were multiple estimates in a study, the effect size that was judged to be the least biased (eg, adjusted for the most confounders, addressed RC and CbI) was extracted for analysis. For gender-stratified studies, effect sizes of boy and girl were extracted respectively. For indication-stratified studies, effect size of antibiotic prescribing for non-respiratory infection was extracted.

Statistical analysis

Meta-analysis was conducted using STATA 15.0 to generate pooled ORs. When HRs or RRs were provided, we assumed HRs were similar to RRs as previous studies did.[12,15] Since the prevalence/incidence rate of asthma is generally low among included studies, we assumed that ORs approximated the RRs.[16,17] Heterogeneity was assessed by Cochrane Q test ($P < 0.05$ indicated statistically significant) and Higgins I^2 test (I^2>50% and I^2>75% indicated moderate and high heterogeneity, respectively). Due to the detected heterogeneity, random effect models were used for all analyses. Random effect models allow both within-study and between-study variances, and are more conservative.[18] We conducted the following analyses to evaluate the association of infant antibiotic exposure and childhood asthma: 1) An overall analysis including the least biased estimates of all studies and stratified analyses by measurement of exposure and outcome, study design type, HR/RR/OR, and country/region; 2) Subgroup analyses based on the adjustment for the common factors; 3) Analysis exclusively for studies that addressed RC and CbI. Studies that additionally adjusted for common factors were further included in a restricted analysis; and 4) Detailed analyses regarding antibiotic type, course, timing, asthma definition, phenotype, age of onset, and time lag between exposure and asthma onset. Antibiotic course refers to the frequency of interval antibiotic prescription within the defined exposure time. To describe a dose-response relationship between antibiotic course and risk of childhood asthma, we assigned a value to each antibiotic course category using the midpoint of category (eg, value of 1.5 for the category of 1–2 antibiotic course) or 1.5 times the lower limit of open-ended category (eg, 7.5 for the category of $\geq$5 course of antibiotic) to fit a linear or non-linear model.[19]

Meta-regression was performed for region, publication year, study type and quality, sex ratio, exposure timing, outcome definition, asthma onset age, measurement, measures of effect size, and common confounders that were used as stratified factors in the results below (eg, RC/CbI) to identify sources of heterogeneity. Sensitivity analysis was performed to examine the stability of the pooled results by removing one study each time and assessing the change of the pooled estimates. Publication bias was evaluated using Begg's and Egger's test ($P < 0.10$ was considered significant). Funnel plot was also presented. Study quality was assessed using the Newcastle–Ottawa Quality Assessment Scale (NOS) for cohort and case-control studies and Agency for Healthcare Research and Quality scales for cross-sectional studies. Although some items of NOS has been questioned[20] and a ROBINS-I tool is recommended for non-randomized studies,[21] we choose NOS in this review because it can be simply applied to assess the quality of case-control studies and cohort studies separately. By comparison, NOS almost covered the range of bias assessed by ROBINS. The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) criteria were also used to assess the quality of evidence for studies that addressed RC and CbI on the following domains: risk of bias, inconsistency, imprecision, indirectness, and publication bias.[22]

RESULTS

Literature search

We identified 1521 non-duplicate articles. After applying the inclusion and exclusion criteria, 112 articles were reviewed in detail, of which 47 articles

were eligible for the present meta-analysis [23-69] (Fig. 1). Among these articles, 5[25,39,46,56,60] included 2 separate studies in the same article. As a result, totally 52 studies out of 47 articles involving 2 742 140 patients were included. The study by Gupta et al[49] met the exclusion criteria by exceeding age of outcome limits (0–21 years), but it was retained because its calculated mean age was 6 years.

Study characteristics

The characteristics of the included studies were summarized in Table S1. Most exposure was limited to the first 2 years, with a wider range of asthma onset age (1–21 years). The majority of studies were prospective studies (n = 22) and adopted information measurement of self-report (n = 32). All included studies reported adjusted effect size except for 2 studies.[47,66] Quality scores of the included studies were presented in Table S1 and displayed in Fig. S1. Overall, 21 studies were of medium quality and 31 studies were of high quality.

Association of infant antibiotic exposure and childhood asthma

The overall analysis pooling from the least biased estimates of all studies showed that infant antibiotic exposure was significantly associated with childhood asthma (OR, 1.37; 95% CI, 1.29–1.45; I^2 = 97.4%, P < 0.01) (Table 1). Cross-sectional studies pooled a higher OR (1.48; 95% CI, 1.33–1.64, I^2 = 45.0%, P = 0.04), and was followed by retrospective studies (OR, 1.37; 95% CI, 1.27–1.47, I^2 = 87.4%, P < 0.01). Prospective studies indicated the lowest but still significant pooled OR (1.32; 95% CI, 1.22–1.43, I^2 = 96.6%, P < 0.01).

We further stratified studies by adjustment for common confounders (Table 1). The pooled OR in the subgroup adjusted for medical consultation (OR, 1.16; 95% CI, 1.00–1.35), CbI (OR, 1.29; 95% CI, 1.19–1.41), sex, birth weight and delivery mode (OR, 1.23; 95% CI, 1.15–1.32), maternal antibiotic exposure (OR, 1.25; 95% CI, 1.08–1.46), RC (OR, 1.34; 95% CI, 1.25–1.44), sex, smoke exposure and parental allergy (OR, 1.35; 95% CI, 1.22–1.50) and studies of high quality (OR, 1.30; 95% CI, 1.22–1.38) showed lower estimates. However, the association of infant antibiotic exposure and childhood asthma still remained in all subgroups.

Confounding by RC and CbI

Studies that addressed RC and CbI pooled an attenuated but statistically significant association

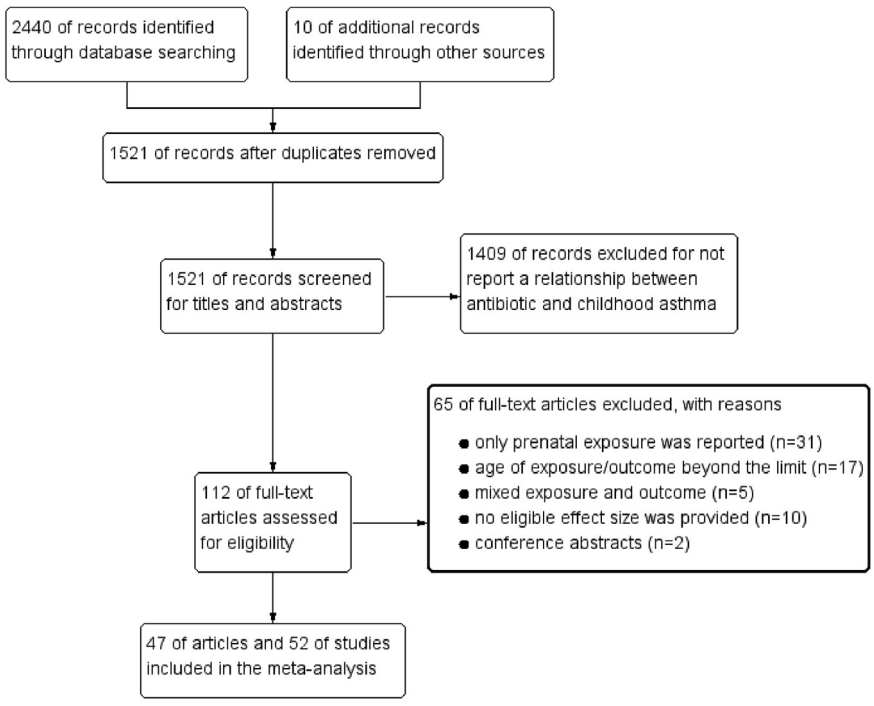

Fig. 1 Flow diagram of selecting studies.

Subgroup types	Number of studies(n)	Heterogeneity		Pooled OR (95%CI)
		I^2 (%)	*P*-value	
Overall analysis	52	97.4	<0.01	1.37 (1.29–1.45)
OR	38	95.6	<0.01	1.44 (1.31–1.59)
RR	3	99.5	<0.01	1.16 (0.85–1.59)
HR	11	95.0	<0.01	1.33 (1.21–1.46)
Measurement of exposure and diagnosis				
Database[a]	20	98.8	<0.01	1.33 (1.22–1.44)
Self-report	32	86.4	<0.01	1.45 (1.32–1.59)
Study design type[b]				
Prospective studies	22	96.6	<0.01	1.32 (1.22–1.43)
Database[a]	10	98.1	<0.01	1.32 (1.20–1.46)
Questionnaire	12	64.8	<0.01	1.38 (1.16–1.64)
Retrospective studies	11	87.4	<0.01	1.37 (1.27–1.47)
Database[a]	8	89.6	<0.01	1.36 (1.21–1.53)
Questionnaire	3	63.3	0.07	1.41 (1.29–1.54)
Cross-sectional studies	14	45.0	0.04	1.48 (1.33–1.64)
Study country/region				
US	12	99.2	0.01	1.34 (1.17–1.52)
Europe	23	95.5	<0.01	1.36 (1.24–1.50)
Asia	12	75.0	<0.01	1.38 (1.24–1.54)
Other[c]	5	37.4	0.17	1.47 (1.22–1.77)
Adjustment for RC				
Yes	35	98.0	<0.01	1.34 (1.25–1.44)
No	17	83.6	<0.01	1.47 (1.29–1.68)
Adjustment for CbI				
Yes	27	97.9	<0.01	1.29 (1.19–1.41)
No	25	77.9	<0.01	1.43 (1.35–1.53)
Adjustment for gender, smoke exposure, and parental allergy				
Yes	13	68.3	<0.01	1.35 (1.22–1.50)
No	39	96.5	<0.01	1.36 (1.27–1.46)
Adjustment for gender, birth weight, and delivery mode				
Yes	10	89.5	<0.01	1.23 (1.15–1.32)
No	42	95.9	<0.01	1.41 (1.31–1.51)
Adjustment for maternal antibiotic exposure				
Yes	6	90.7	<0.01	1.25 (1.08–1.46)
No	46	97.6	<0.01	1.39 (1.30–1.48)
Adjustment for medical consultation				
Yes	8	94	<0.01	1.16 (1.00–1.35)
No	44	97.4	<0.01	1.42 (1.33–1.51)
Quality[d]				
High	31	97.3	<0.01	1.30 (1.22–1.38)
Medium	21	97.5	<0.01	1.56 (1.29–1.89)

Table 1. The overall analysis and subgroup analyses of childhood asthma risk associated with infant antibiotic exposure. *Abbreviations: OR, odds ratio; CI, confidence interval; RC, reverse causation; CbI, confounding by indication. a. Database studies often define a asthma diagnosis using a combination of International Classification of Diseases code and drug prescription records. b. As 2 studies did not report adjusted effect size,[47,66] 1 study included adult patients,[49] and 1 study involved premature infants,[35] these studies were excluded in the first subgroup analysis to reduce the sources of heterogeneity. c. Other countries/regions included Angola, Costa Rica, Cuba, Brazil, and Canada. d. For cohort studies and case-control studies, score of 4–6 was considered as medium quality and 7–9 as high quality. For cross-sectional studies, score of 4–7 was considered as medium quality and 8–11 as high quality*

Subgroup types	Number of studies(n)	Heterogeneity		Pooled OR (95%CI)
		I^2 (%)	*P*-value	
Studies that addressed RC and CbI	23	95.3	<0.01	1.19 (1.11–1.28)
OR	15	57.6	0.01	1.14 (1.06–1.23)
RR	2	94.8	<0.01	1.25 (0.86–1.82)
HR	6	87.2	<0.01	1.20 (1.09–1.32)
Study type				
Prospective studies	12	97.4	<0.01	1.24 (1.12–1.37)
Retrospective studies	7	77.7	<0.01	1.15 (1.00–1.32)
Cross-sectional studies	4	0.0	0.60	1.11 (0.99–1.24)
Measurement of exposure and diagnosis				
Database	12	97.6	<0.01	1.19 (1.09–1.30)
Self-report	11	43.7	0.06	1.21 (1.06–1.37)
Study country/region				
US	8	55.7	0.03	1.16 (1.07–1.25)
Europe	9	95.9	<0.01	1.16 (1.02–1.33)
Asia	5	72.8	<0.01	1.30 (1.04–1.61)
Adjustment for maternal antibiotic exposure				
Yes	4	74.4	<0.01	1.11 (1.01–1.23)
No	19	96.1	<0.01	1.22 (1.12–1.34)
Adjustment for gender, smoking, and parental allergy				
Yes	6	22.5	0.27	1.18 (1.15–1.21)
No	17	91.3	<0.01	1.18 (1.07–1.30)
Adjustment for gender, birth weight, delivery mode				
Yes	5	67.1	0.02	1.15 (1.11–1.20)
No	18	87.2	<0.01	1.22 (1.12–1.34)

Table 2. The particular analysis and restricted analysis for studies that addressed RC and CbI. *Abbreviations: OR, odds ratio; CI, confidence interval; RC, reverse causation; CbI, confounding by indication. Database studies often define a asthma diagnosis using a combination of International Classification of Diseases code and drug prescription records.*

(1.19; 95% CI, 1.11–1.28; $I^2 = 95.3\%$, $P < 0.01$) (Table 2) (Fig. S2). To further analyze whether this significance was biased by other confounding, we restricted studies to those additionally adjusting for common factors. Results indicated that studies additionally adjusting for maternal antibiotic exposure (OR, 1.11; 95% CI, 1.01–1.23), sex, birth weight and delivery mode (OR, 1.15; 95% CI, 1.11–1.20), and sex, smoke exposure and parental allergy (OR, 1.18; 95% CI, 1.15–1.21) had lower risk. When stratified by study type, the association remained in prospective studies (OR, 1.24; 95% CI, 1.12–1.37, $I^2 = 97.4\%$, $P < 0.01$), while almost disappeared in retrospective studies (OR, 1.15; 95% CI, 1.00–1.32, $I^2 = 77.7\%$, $P < 0.01$) and disappeared in cross-sectional studies (OR, 1.11; 95% CI, 0.99–1.24, $I^2 = 0.0\%$, $P = 0.60$).

In the overall analysis and the RC, CbI-adjusted analysis, the subgroup of studies reporting RR lost the significance (Tables 1 and 2). However, significant association maintained across all subgroups stratified by exposure/outcome measurement and study country/region, with a trend of stronger association observed for studies using questionnaires and studies conducting in Asia (Tables 1 and 2).

Antibiotics: type, course, and exposure timing

Penicillin was the most frequently prescribed antibiotic type among included studies, with the exception of cephalosporin being most commonly used in Japan (Table S1). Macrolides were associated with a greater risk of developing asthma (OR, 1.56; 95% CI, 1.31–1.86) than

Subgroup types	Number of studies(n)	Heterogeneity		Pooled OR (95%CI)
		I^2 (%)	*P*-value	
Antibiotic type				
Penicillin	10	97.4	<0.01	1.33 (1.15–1.54)
Cephalosporin	11	98.0	<0.01	1.39 (1.20–1.62)
Macrolides	11	97.8	<0.01	1.56 (1.31–1.86)
Antibiotic course				
1-2	15	94.7	<0.01	1.29 (1.18–1.42)
3-4	14	97.8	<0.01	1.79 (1.49–2.14)
≥5	8	98.4	<0.01	1.79 (1.36–2.36)
Antibiotic exposure timing				
1 week	3	0.0	0.61	1.82 (1.34–2.47)
6 months	10	98.9	<0.01	1.43 (1.25–1.63)
6 months-1 year	6	63.3	0.02	1.31 (1.14–1.50)
1 year	11	72.0	<0.01	1.41 (1.25–1.59)
2 years	10	59.8	<0.01	1.42 (1.27–1.59)
Adjusted for RC and CbI	5	64.2	0.03	1.25 (1.07–1.47)
Unadjusted for RC and CbI	5	0.0	0.87	1.65 (1.48–1.84)
3 years	5	56.9	0.05	1.54 (1.30–1.82)
Age of asthma onset				
1–3 years	8	95.1	<0.01	1.84 (1.63–2.08)
Adjusted for RC and CbI	3	68.9	0.04	1.83 (1.48–2.28)
Unadjusted for RC and CbI	5	97.0	<0.01	1.83 (1.51–2.22)
4–6 years	11	60.3	<0.01	1.28 (1.18–1.39)
Adjusted for RC and CbI	4	0.0	0.60	1.17 (1.14–1.21)
Unadjusted for RC and CbI	7	51.8	0.05	1.37 (1.20–1.57)
>6 years	4	64.4	0.04	1.11 (0.96–1.28)
Time interval				
0–2 years	7	93.0	<0.01	2.05 (1.91–2.20)
2–5 years	7	89.6	<0.01	1.38 (1.17–1.62)
5–6 years	4	0.0	0.99	1.17 (1.13–1.21)
Asthma definition				
Asthma	41	96.7	<0.01	1.39 (1.30–1.49)
Transient asthma[a]	3	97.2	<0.01	2.46 (1.50–4.04)
Persistent asthma[b]	3	96.7	<0.01	2.61 (1.49–4.59)
Late-onset asthma[c]	3	0.0	0.67	1.11 (1.06–1.15)
Wheezing	7	90.9	<0.01	1.48 (1.14–1.93)
Atopic asthma	4	0.0	0.70	2.14 (1.58–2.90)

Table 3. Detailed analyses regarding antibiotic type, course, exposure timing, age of asthma onset, asthma outcome, and time interval.
Abbreviations: OR, odds ratio; CI, confidence interval; RC, reverse causation; CbI, confounding by indication. a. Transient asthma: began and resolved before 3 years of age. b. Persistent asthma: began before 3 years of age and persisted through to 4–7 years of age. c. Late-onset asthma: began after 3 years of age

penicillin (OR, 1.33; 95% CI, 1.15–1.54) and cephalosporin (OR, 1.39; 95% CI, 1.20–1.62) (Table 3). An increased risk of asthma was observed when antibiotic course increased from 1 to 2 (OR, 1.29; 95% CI, 1.18–1.42) to 3–4 (OR, 1.79; 95% CI, 1.49–2.14), and a plateau when increased to 5 courses (OR, 1.79; 95% CI, 1.36–2.36) (Table 3), revealing a non-linear dose-response relationship ($P < 0.01$) (Fig. 2). Subgrouping studies by exposure timing produced different effects (Table 3) (Fig. 3). The strongest effect was observed when exposure was in the first week of life (OR, 1.82; 95% CI, 1.34–2.47). A slightly stronger effect was observed when exposure was in the second (OR, 1.42; 95% CI, 1.27–1.59) and third year (OR, 1.54;

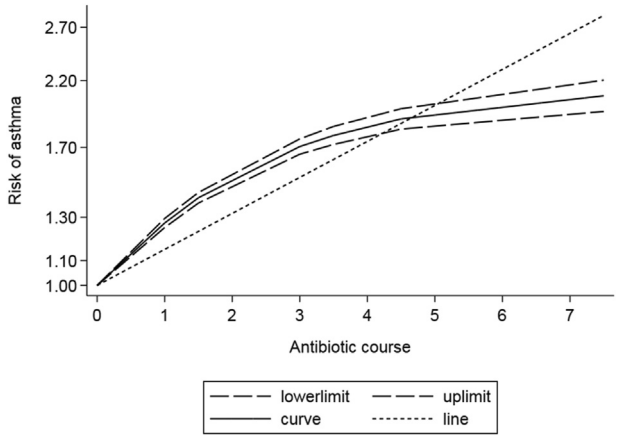

Fig. 2 Linear and non-linear fit model demonstrating a dose-response relationship between antibiotic exposure course and childhood asthma.

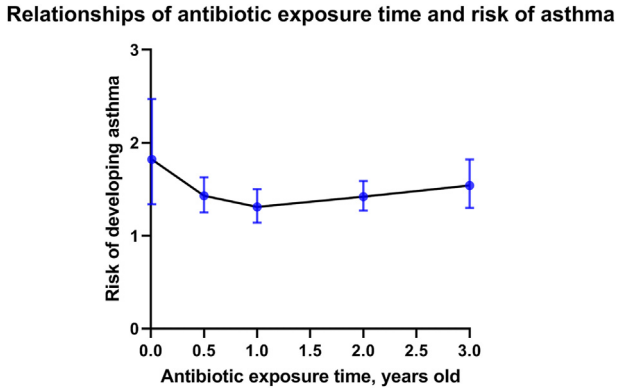

Fig. 3 Association of antibiotic exposure timing and risk of childhood asthma.

95% CI, 1.30–1.82). However, when RC and CbI were considered, reduced effect was observed in the second year (OR, 1.25; 95% CI, 1.07–1.47).

Asthma: age of onset, time interval and definition

Association of infant antibiotic exposure and risk of childhood asthma was strong at younger age (OR for 1–3 years, 1.84; 95% CI, 1.63–2.08) and became insignificant at >6 years old (OR, 1.11, 95% CI, 0.96–1.28) (Table 3) (Fig. 4). Reduced effect and heterogeneity was observed in the subgroups that adjusted for RC and CbI. Besides, the longer time interval between exposure and asthma onset, the lower risk of asthma was reported (OR for 0–2years, 2.05, 95% CI, 1.91–2.20 and OR for 5–6years, 1.17, 95% CI, 1.13–1.21). Pooling studies using definite asthma diagnosis showed similar pooled OR (1.39; 95% CI, 1.30–1.49) to that of the overall analysis. Significantly higher pooled OR appeared in studies reporting wheezing (OR, 1.48; 95% CI, 1.14–1.93), persistent asthma (OR, 2.61; 95% CI, 1.49–4.59), and atopic asthma (OR, 2.14; 95% CI, 1.58–2.90) (Table 3).

Meta-regression analysis, sensitivity analysis, publication bias and GRADE evidence

Meta-regression analysis showed that study quality (P = 0.02), adjustment for medical consultation (P = 0.02), and adjustment for RC and CbI (P = 0.04) could be the sources of heterogeneity.

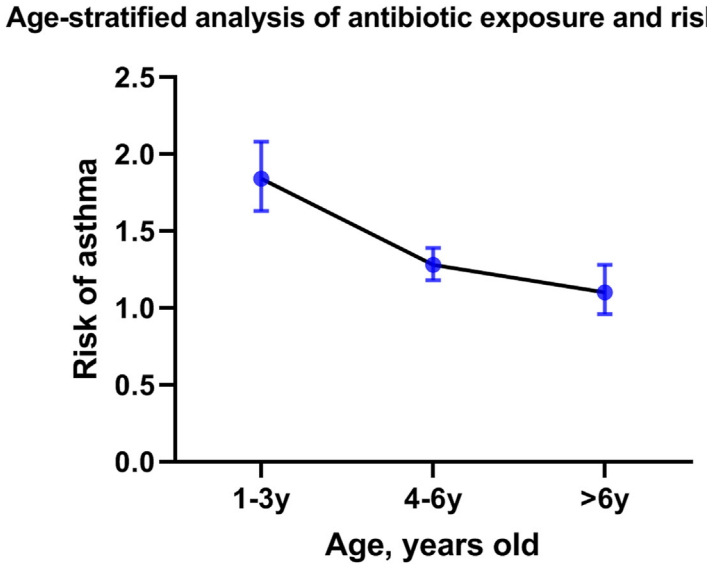

Fig. 4 Age-stratified analysis of antibiotic exposure and risk of asthma.

Sensitivity analysis suggested robustness of our findings (Fig. S3). Begg's and Egger's test did not detect significant publication bias (P = 0.61 and P = 0.17, respectively). Funnel plot showed an asymmetry among small studies, suggesting that there may be some small study effect (Fig. S4). According to the GRADE criteria, the quality of evidence for studies that addressed RC and CbI was low due to study design and heterogeneity identified among studies (Table S2).

DISCUSSION

In this meta-analysis, we showed that infant antibiotic exposure was associated with development of childhood asthma. Correction for RC, CbI, and other common confounders attenuated the association but did not change the significance. In the detailed analyses, macrolides, course of 3–4, exposure within 1 week of birth, asthma developed among 1–3 years, short time interval, persistent asthma, and atopic asthma phenotype had greater effect. The heterogeneity among studies and the low value of the GRADE assessment called for caution when consider the results.

Infant antibiotic exposure has been associated with childhood asthma development in recent decades. However, whether this association is biased by other influential factors is worth considering. Acknowledged factors among studies include sex, smoke exposure, familial allergy, birth weight, and delivery mode. Additionally, maternal antibiotic exposure during pregnancy may induce childhood asthma by interfering the neonatal microbiota, and thus confounds the effect of infant antibiotic exposure.[70,71] Medical consultation may be a reflection of the susceptibility to diseases under various risk factors[72] and was considered as potential confounder.[13] Pooling studies that adjusted for the above confounders showed that infant antibiotic exposure was still associated with childhood asthma. Besides, RC and CbI are two highlighted factors that strongly confounded the association between infant antibiotic exposure and childhood asthma. In the present analysis, studies that addressed RC and CbI showed an increased asthma risk of 18% when exposed to infant antibiotic with a narrower 95%CI, which indicates a more precise estimate. This result is in line with a meta-analysis including non-retrospective studies that addressed RC and CbI, which showed a small but significant association.[12] When we restricted studies to those that addressed RC, CbI and other fore-examined confounders, statistical significance remained. These findings indicate that the association between infant antibiotic and childhood asthma was relatively stable and only partly biased by these confounders.

Prospective studies with full consideration for RC and CbI are expected to provide casual inferences. In this meta-analysis, significant association remained in prospective studies that addressed RC and CbI, supporting a possible cause-and-effect relationship between antibiotics and asthma. However, this result is inconsistent with previous meta-analyses.[12,13] This discrepancy may be due to the short antibiotic exposure window of those studies, which can underestimate the true effect of infant antibiotic because exposure occurred in the second or third year of life also associated with increased risk of subsequent asthma.[23,37,47] Besides, over the past decade, increased access to medical counseling has led to more asthma detection.[73] Reduced exposure to farms or fields lead to reduced microbio-diversity in children.[74] In view of the above reasons, a causal effect of infant antibiotic on risk of childhood asthma in this study, or at present, seems to be rational. If there is a true causality, attention should be drawn to reduce unnecessary antibiotic prescription given that the risk of developing asthma increased by 24% when exposed to infant antibiotic and a fact that a huge number of children prescribed antibiotics.

The association between infant antibiotic exposure and childhood asthma can be elucidated by the microbiota hypothesis. Microbiota plays a key role in the immune modulation by the function of protecting from pathological bacteria, differentiating lymphocytes, and promoting Th1 phenotype development.[75] Antibiotic exposure can reduce the airway and gastrointestinal microbiome diversity,[6] affect the balance between microbiota and immune system, and thus increase the risk of asthma development. In view of that, antibiotic type, course, and exposure timing may all modify the association through microbiota-mediated mechanism. Based on the previous studies, broad-spectrum antibiotics (e.g. penicillin,

cephalosporin, macrolides) tend to show greater effect,[66,76] among which macrolides showed the greatest pooled effect in the present meta-analysis. This result can be supported by the finding that macrolides cause greater disorder to the microbiome.[77] Regarding antibiotic course, related studies reported that the risk of asthma increased with antibiotic course in a dose-response fashion.[47,51] However, a non-linear relationship was further revealed in the present meta-analysis.[12] This phenomenon has also been reported in a previous meta-analysis with nearly the same pooled estimate for >4 and 3–4 courses. However, explanation was not given in that study. We infer that this nonlinear relationship may be due to the "antibiotic resistance" of microbiome, meaning that microbiome may be more sensitive to preliminary antibiotic exposure.[78,79] This hypothesis needs to be elucidated in future studies. The first 6 months after birth are crucial for the development of a healthy microbiome.[7] Antibiotics exposed in this period can profoundly affect the microbiome composition[80] and lead to increased risk of asthma, which is accordant with our results that exposure in the first week of life and in the first 6 months of life showed a relatively high risk. Unexpectedly, risk was higher when exposure at the second and third years than at the first year, which possibly arises from the cumulative effect of antibiotics. Besides, when studies limited their exposure window to the first year of life, effect of antibiotics exposed within the first year could be underestimated by classifying children who received antibiotics in their second and third year as unexposed children. Increased incidence of RC and CbI in later exposure may also account for it, as the effect of exposure at the second year reduced when adjusted for RC and CbI.

The role of infant antibiotic exposure in childhood asthma varied with age of asthma onset. We observed a stronger effect at age of 1–3 years, while an insignificant effect at age ≥6 years. Besides the high incidence of RC at early age, reason for the result may be that antibiotics may exert the greatest effect on microbiome shortly after the exposure.[56,81] This is supported by the time interval-stratified analysis that a higher risk of asthma onset is observed on a short time scale after exposure. These findings underscore the importance of protecting microbiota and preventing asthma following early-life antibiotic exposure and in the early stage after exposure. Besides, an accurate diagnosis of asthma for children younger than 3 years old is difficult[51] and is often confused with wheezing. Wheezing is common in infants and mostly disappears with age,[82] and wheezing from a RI is often treated with antibiotics,[83] both of which lead to an overestimated effect in studies using "wheezing" as outcomes. We also observed a high pooled estimate for studies analyzing atopic asthma. Atopic asthma and non-atopic asthma are 2 phenotypes of childhood asthma, which have different risk factors.[84] Non-atopic asthma seems to be more related to early infection and genetics.[85] Current studies mostly linked antibiotics with various childhood atopic diseases,[86,87] and thus antibiotics may have greater roles in atopic asthma. Similarly increased risk was observed in persistent asthma phenotype, although with a broad 95%CI due to small sample size. This significance became understandable when considering the long-standing and irreversible damage to the development of microbiota and subsequent immune system caused by antibiotic exposure in the childhood.[88,89] Future studies are expected to detail the association of infant antibiotic with asthma phenotype and how it varies with age, because this will help with risk factor identification and prevention of different asthma phenotype.

There is high heterogeneity among included studies. In addition to stratified analyses and meta regression on confounding factors, we also examined the assumption that HR, RR, OR are similar. Although the pooled estimates of studies using HR and OR were consistent, the subgroup of RR lost its significance. We think this may be due to the small number of included studies and the great heterogeneity in this subgroup. Therefore, caution is still required to interpret the findings. Besides, we also conducted stratified analyses by region. Significant association maintained among subgroups, with higher estimates reported in subgroup of Asia. This may contradict to our hypothesis, as the prevalence of asthma for Asian was reported lower than that for Americans or Europeans.[2,90] However, studies also showed that compared with White, South Asian exposed to

infant antibiotic had higher risk of childhood asthma development, while not for East Asian.[26] Future studies examining this association among different regions and ethnicities are required.

Compared with previous meta-analyses, our findings are extensive and novel in that more influential factors were considered and combined with RC and CbI to analyze the effect of confounding. Besides, our study extended antibiotic exposure window to the second and third year. Accordingly, to avoid the confounding by RC, we conducted a series of stratified analyses. Furthermore, our detailed analyses of different settings of exposure and outcome can help to understand the association in depth, as well as provide clinical implications for asthma prevention. However, limitations include the heterogeneity among studies, pooling studies collectively using ORs, and again, caution is required when interpret the results. Besides, different adjusted models were adopted among the included studies and it was not possible to take into account all the confounders together in one subgroup analysis. Additionally, there are still several confounders, such as air pollution and socio-economic status that were not examined in the present analysis. Finally, underestimated effect of antibiotic cannot be ruled out since the actual antibiotic exposure may be overestimated in database studies that using prescriptions filled as the measurement of exposure.

CONCLUSIONS

This meta-analysis indicated that infant antibiotic exposure is associated with an increased risk of childhood asthma. Common confounders could only explain part of the association. This study further supports the reduction of unnecessary antibiotic prescription during infancy, and underlines the importance of asthma prevention in the early stage following early-life antibiotic exposure. Future studies into the effect of antibiotic on different asthma phenotypes are required, and caution is needed when interpret the results.

Abbreviations

OR, odds ratio; RR, relative risk; HR, hazard ratio; CI, confidence interval; RC, reverse causation; CbI, confounding by indication; BMI, body mass index; NICU, neonatal intensive care unit; NOS, Newcastle–Ottawa Quality Assessment Scale; AHRQ, Agency for Healthcare Research and Quality; RI, respiratory infection; GRADE, the Grades of Recommendation, Assessment, Development and Evaluation; PRISMA, the Preferred Reporting Items for Systematic Reviews and Meta-Analysis.

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Ethical approval of studies

Not applicable.

Informed consent

Not applicable.

Authors' consent for publication

All authors have reviewed and consented to publication.

Data availability statement

All information can be available in the paper.

Author contribution

Jingjing Wang searched the articles. Haixia Wang, Yizhang Li, Mo Yi and Yuanmin Jia assessed the article quality. Zeyi Zhang was a major contributor in writing the manuscript. Ou Chen is responsible for review and modification of the manuscript.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article to disclose.

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Not applicable.

Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.waojou.2021.100607.

Author details

School of Nursing and Rehabilitation, Cheeloo College of Medicine, Shandong University, #44 West Wenhua Road, Jinan, 250012, China.

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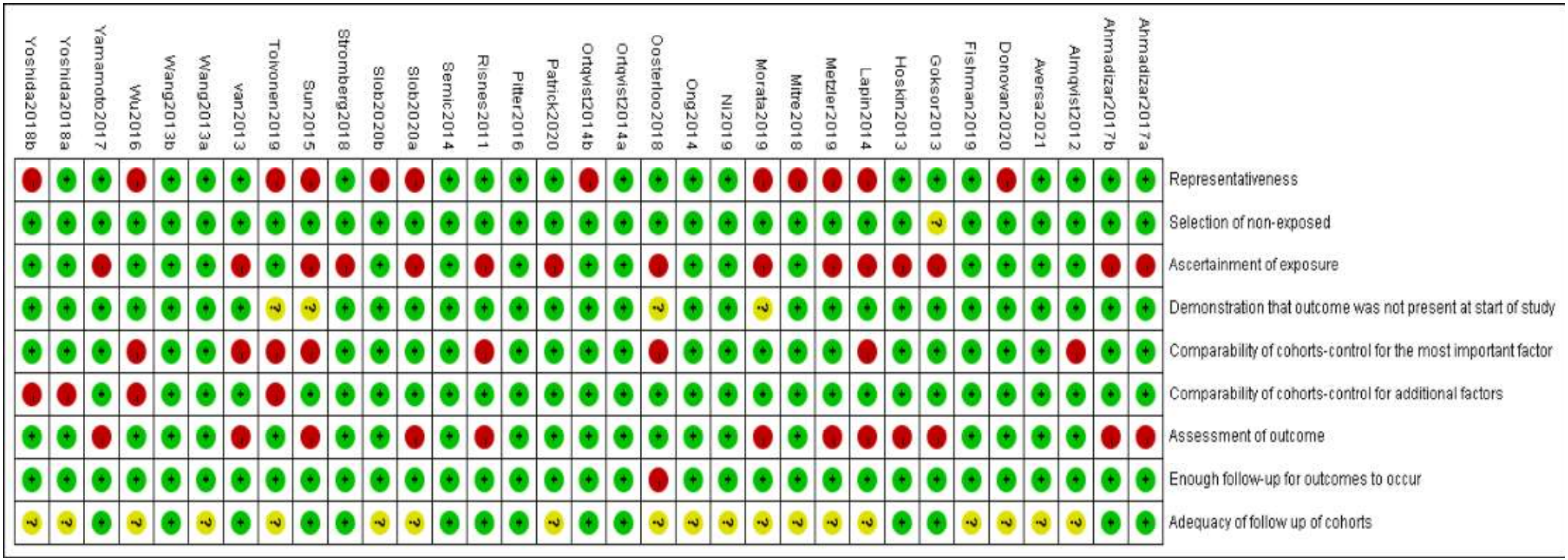
Supplementary figures:

A



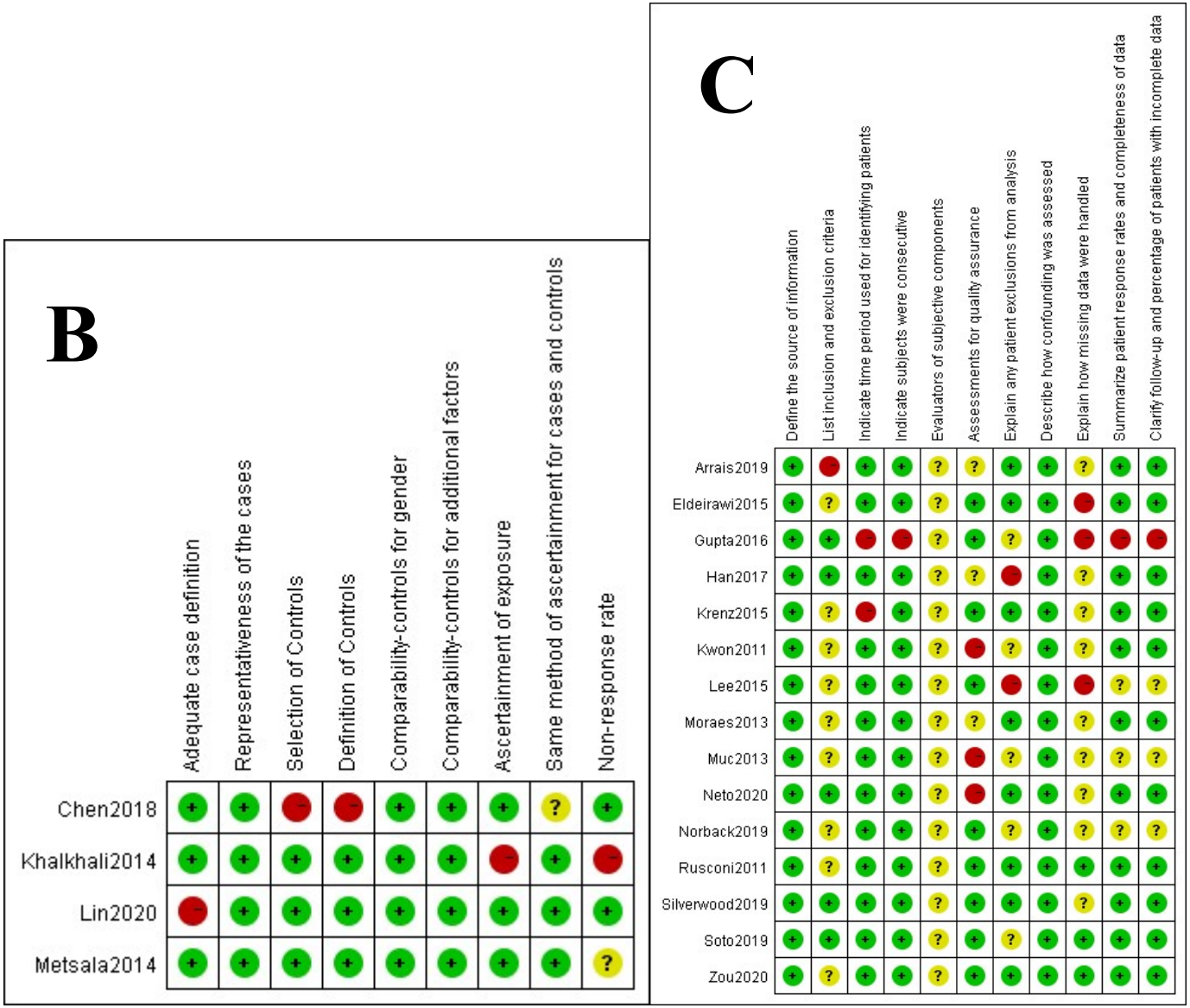


Figure S1 Details about quality assessment for included studies. (A) Cohort studies assessed by Newcastle–Ottawa Quality Assessment Scale. (B) Case-control studies assessed by Newcastle–Ottawa Quality Assessment Scale. (C) Cross-sectional studies assessed by Agency for Healthcare Research and Quality scales.

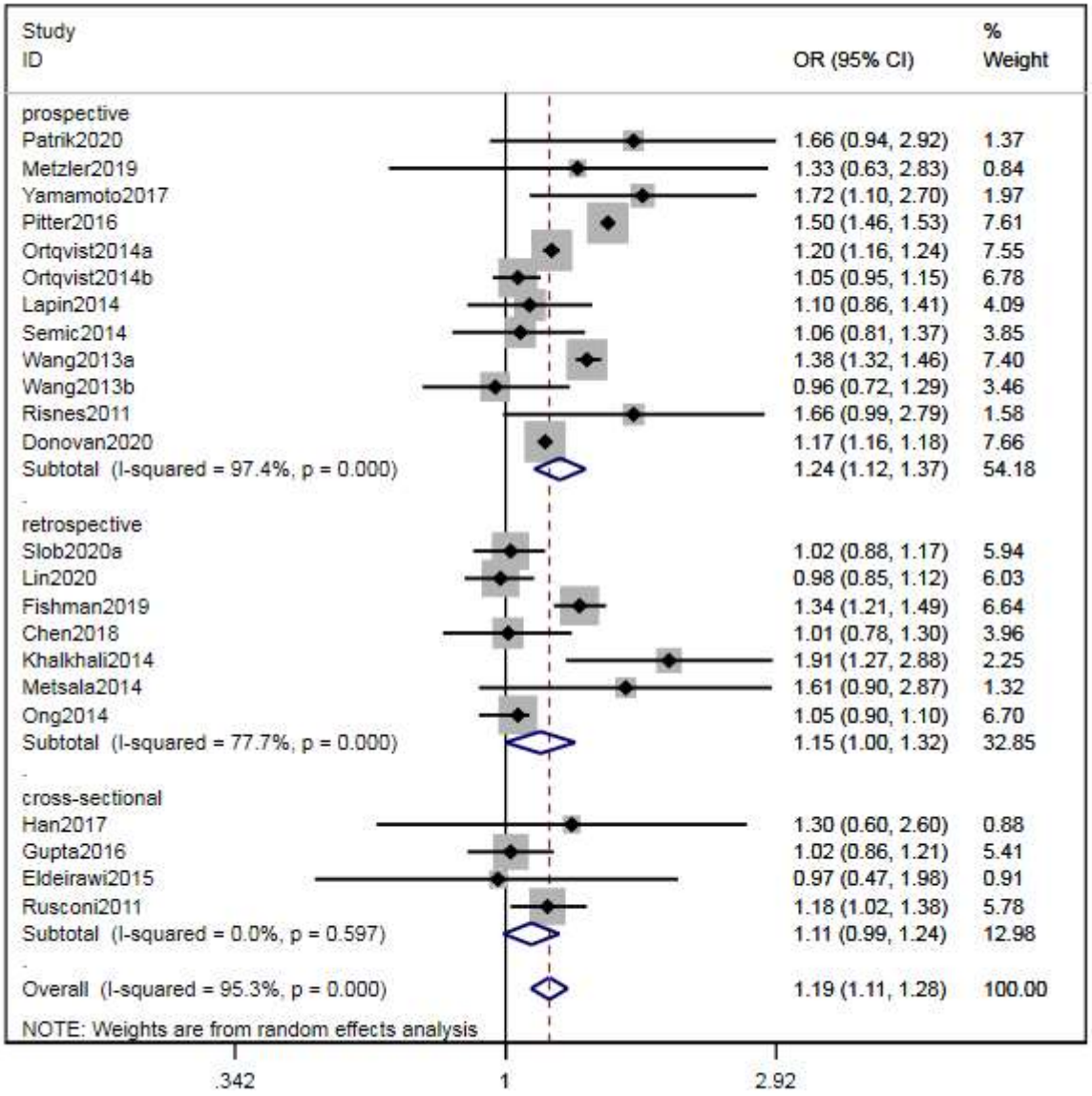


Figure S2 Analysis for studies that addressed RC and CbI

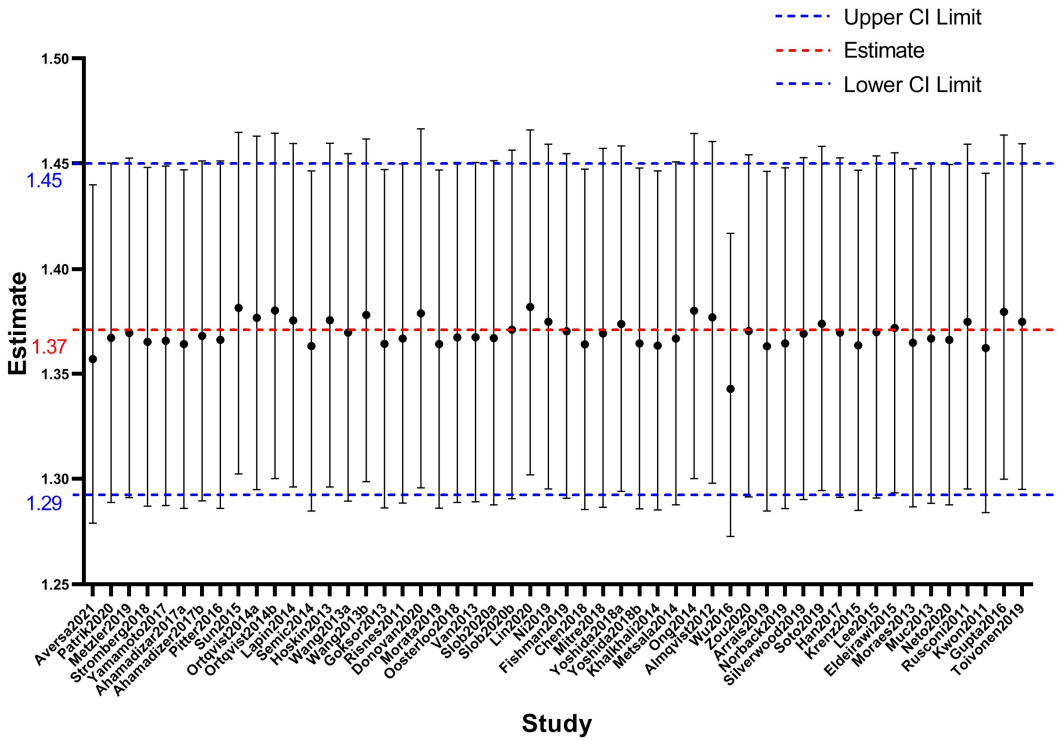


Figure S3 Sensitivity analysis for the overall analysis

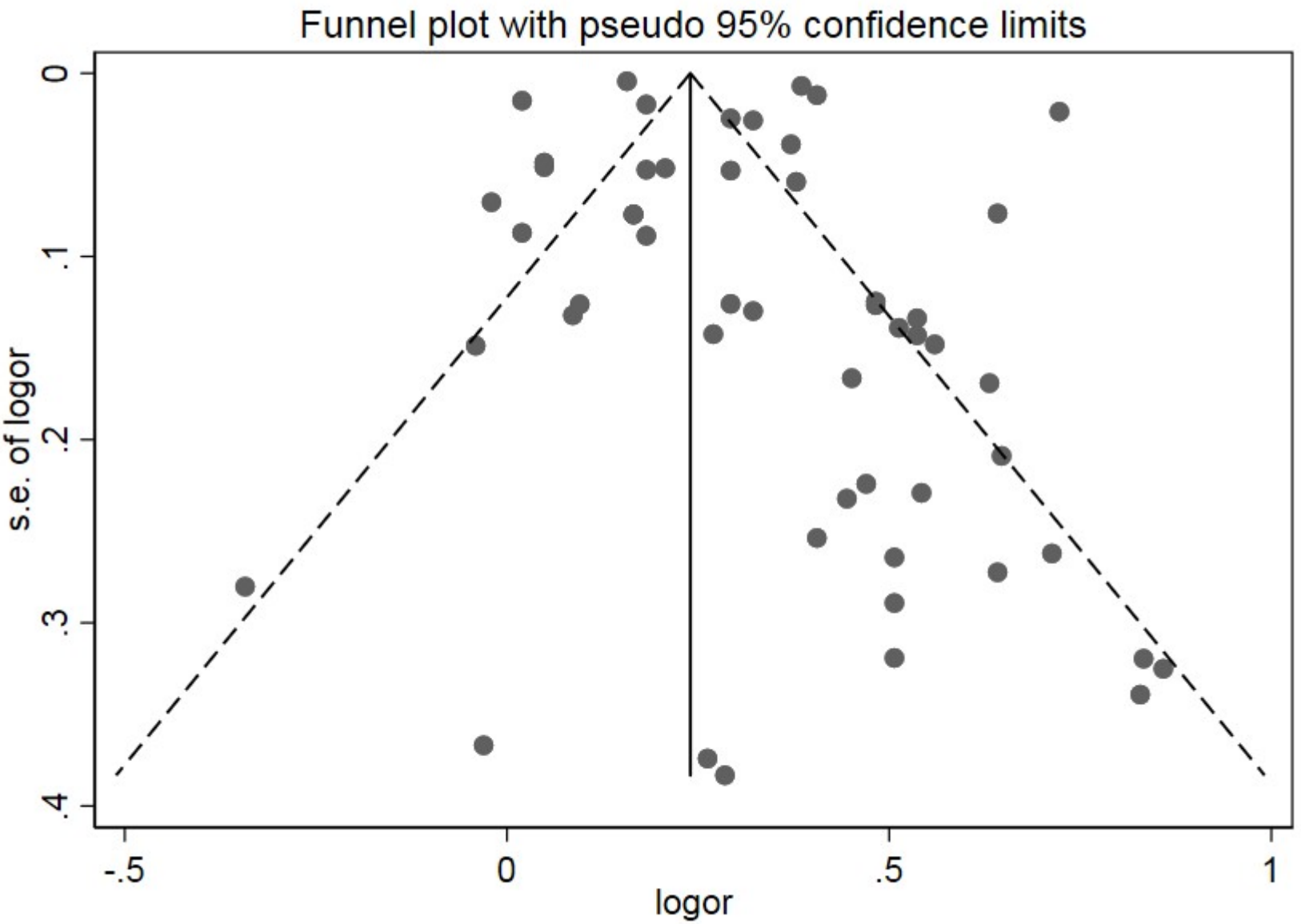


Figure S4 Funnel plot of the overall analysis

Table S1 Characteristics of included studies in this meta-analysis

Study	Study design	Sample size	Exposure timing	Asthma age	Measurement	Effect size, 95%CI	RC	CbI	Adjusted model‡	Quality score[a]
Aversa 2021	Prospective	14572	0-2 years	2-14 years	Database	HR 1.90(1.63-1.22)	Yes	No	Sex, ethnicity, delivery mode, maternal age, education, smoking, antibiotic exposure during pregnancy	8 High
Zou 2020	Cross-sectional	13335	0-1 year	4-6 years	Questionnaires	OR 1.31(0.99-1.73)	No	Yes	Child's age, sex, district, family allergy, breastfeeding, early home decoration, pet-keeping, smoke, and home dampness-related exposures	9 High
Patrick 2020	Prospective	2644	0-1 year	5 years	Questionnaires	OR 1.66(0.94-2.92)	Yes	Yes	Ethnicity, gender, sibling, delivery mode, birth weight, breastfeeding, season of birth, smoking, parental allergy, No_2 exposure	7 High
Slob 2020	Retrospective	34352	0-2 years	3-12 years 9 years†	Questionnaires	OR† 1.45(1.34-1.56) 1.34(1.28-1.41)	Yes	Yes	Gender, day care, breastfeeding, delivery mode, birth weight, parental education	7High 5Medium †
Lin 2020	Retrospective	3236	0-3 years	4-12 years	Database	OR 0.98(0.85-1.12)	Yes	Yes	Age, gender, resident urbanization, comorbidities and medication	8 High
Donovan 2020	Prospective	152622	0-6 months	4.5-6 years	Database	OR 1.17(1.16-1.18)	Yes	Yes	Smoking, maternal age, maternal education, marital status, residence, gestational age, delivery mode, siblings, birth weight, infant race, sex	7 High
Fishman 2020	Retrospective	79582	0-2 years	2.5-5 years	Database	HR 1.34(1.21-1.49)	Yes	Yes	Gender, birth year, residence, preterm, pre-index autoimmune condition, No. of pre-index outpatient visits, maternal allergy, delivery mode	8 High
Neto 2020	Cross-sectional	1003	0-6 months	9-12 years	Questionnaires	OR 1.57(1.13-2.17)	No	Yes	Bronchiolitis, rhinitis, pet, maternal asthma, paracetamol	8 High
Morata 2019	Prospective	232	0-3 years	7-8 years	Questionnaires	OR 2.36(1.25,4.47)	No	No	Parental allergy, sibling, city environment, infant bronchiolitis, ethnicity, maternal age, birth weight, day care, breastfeeding, smoking, weight gain	4 Medium

Table S1 (Continued)

Study	Study design	Sample size	Exposure timing	Asthma age	Measurements	Effect size, 95%CI	RC	CbI	Adjusted model	Quality score
Toivonen 2019	Prospective	923	0-2 years	7.5 years	Database	OR 1.2(1.09-1.34)	No	Yes	Sex, birth by cesarean section, child's atopy at age 13 months, parental asthma, and parental smoking	4 Medium
Ni 2019	Retrospective	2398	0-1 year	10 years	Database	OR 1.18(1.02-1.38)	Yes	No	Age, sex, ethnicity, socioeconomic status, delivery method, NICU status, birthweight, and prematurity	8 High
Arrais 2019	Cross-sectional	3080	0-1 year	6-7 years	Questionnaires	OR 1.75(1.31-2.34)	No	No	Rhinitis, rash, cooking, conditioner, paracetamol, breastfeeding, sibling, pet, traffic, smoking	7 Medium
Norback 2019	Cross-sectional	39782	0-1 year	3-6 years	Questionnaires	OR 1.62(1.26-2.07)	No	Yes	Gender, age, season of birth, birth weight, breastfeeding, parental allergy, smoking, residence, traffic, city PM_{10}, urban	5 Medium
Metzler 2019	Prospective	1019	0-1 year	3-6 years	Questionnaires	OR 1.33(0.63-2.83)	Yes	Yes	Farm, parental allergy, gender, smoking, siblings, pets, caesarean section, maternal education	5 Medium
Silverwood 2019	Cross-sectional	131924	0-1 year	6-7 years	Questionnaires	OR 1.38(1.07-1.78)	No	No	Maternal education, birth weight, breastfeeding, pet, traffic, farm, fast food, smoking, cooking	9 High
Soto 2019	Cross-Sectional	2817	0-1 year	6-13 years	Questionnaires	OR 1.2(1.01-1.43)	No	No	Comorbidities, paracetamol	9 High
Chen 2018	Retrospective	4164	0-3 year	2-18 years	Database	OR 1.67(1.27-2.19)	Yes	Yes	Age, gender, resident urbanization, other comorbidities, medication	6 Medium
Mitre 2018	Retrospective	792130	0-6 months	3.7 years	Database	HR 1.47(1.45-1.49)	Yes	No	Preterm, delivery mode, gender, medication	7 High
Yoshida 2018	Retrospective	83470	0-1 year	1-6 years	Database	HR† 1.23(1.11-1.36) 1.62(1.27-2.07)	Yes	No	Gender, genetic(familial factors)	7High 6Medium†
Stromberg 2018	Prospective	233	0-1 week	12 years	Questionnaires	OR 1.90(1.10-3.20)	Yes	No	Smoking, social status, preterm, birth weight, breastfeeding, education, gender, medication during pregnancy, pet, familial allergy	8 High
Oosterloo 2018	Prospective	436	0-1 week	1 year	Questionnaires	OR 1.56(0.99-2.46)	No	No	Breastfeeding, smoking, day care, sibling, parental allergy, parental education	4 Medium

Table S1 (Continued)

Study	Study design	Sample size	Exposure timing	Asthma age	Measurements	Effect size, 95%CI	RC	CbI	Adjusted model	Quality score
Yamamoto 2017	Prospective	1196	0-2 year	5 years	Questionnaires	OR 1.72(1.10-2.70)	Yes	Yes	Maternal allergy, maternal education, maternal age, maternal BMI, maternal smoking, delivery mode, gestational age, sibling, daycare, bronchitis, sex	7 High
Ahamadizar 2017	Prospective	8284	0-3 years 0-6 months†	10 years	Questionnaires	OR† 2.04(1.70-4.75) 1.50(0.91-2.46)	Yes	No	Age, gender, familial asthma/allergy in Generation R; gender, familial asthma in SEATON	7 High
Han 2017	Cross-sectional	1517	0-1 year	6-7 years	Questionnaires	OR 1.30(0.60-2.60)	Yes	Yes	Age, gender, parental allergy, rural residence, paracetamol, bronchitis	7 Medium
Gupta 2016	Cross-sectional	1359	0-1 year	2-21 years	Questionnaires	RR 1.02(0.86-1.21)	Yes	Yes	Hygiene factors, age, race, sex, household income, pets, family history, breastfeeding, child care	4 Medium
Wu 2016	Prospective	136098	0-1 year	4.5-6 years	Database	OR 2.06(1.98-2.15)	Yes	Yes	Not provided	5 Medium
Pitter 2016	Prospective	143163	0-1 year	1-17 years	Database	RR 1.50(1.46-1.53)	Yes	Yes	Year of birth, sex, maternal age, maternal education, birth weight, gestational age, hospital admissions for infections	9 High
Krenz 2015	Cross-sectional	1277	0-1 year	8 years	Questionnaires	OR 1.71(1.29-2.26)	No	No	Breastfeeding, sibling, smoking, BMI	7 Medium
Lee 2015	Cross-sectional	7389	0-1 year	14 years	Questionnaires	OR 1.34(1.05-1.72)	No	Yes	Age, gender, BMI, parental allergy, smoking, household income	5 Medium
Eldeirawi 2015	Cross-sectional	2028	0-1 year	4-18 years	Questionnaires	OR 0.97(0.47-1.98)	Yes	Yes	Age, sex, country, history of ear infections in infancy, having a regular doctor or clinic, parental history of asthma or allergies	8 High
Sun 2015	Prospective	606	0-1 year	3 years	Questionnaires	RR 1.02(0.99-1.05)	No	Yes	Preterm, NICU, parental allergy, maternal education	4 Medium
Ortqvist 2014	Prospective	493785	0-2 years	2-18 years	Database	HR† 1.20(1.16-1.24) 1.05(0.95-1.15)	Yes	Yes	Maternal smoking, parity, family situation, mother's country of birth, parental education, maternal asthma, age, genetics(familial)	9High 8High†

Table S1 (Continued)

Study	Study design	Sample size	Exposure timing	Asthma age	Measurements	Effect size, 95%CI	RC	CbI	Adjusted model	Quality score
Khalkhali 2014[52]	Retrospective	607	0-1 year	2-8 years	Questionnaires	OR 1.91(1.27-2.88)	Yes	Yes	Age, gender, delivery mode, parity, preterm, familial allergy	7 High
Lapin 2014	Prospective	300	0-1 year	3 years	Questionnaires	OR 1.10(0.86-1.41)	Yes	Yes	Ethnicity, maternal smoking, antibiotics and ibuprofen during pregnancy, maternal asthma	4 Medium
Semic 2014	Prospective	961	0-2 year	1-11 years	Database	HR 1.71(1.32-2.23)	Yes	Yes	Gender, breastfeeding, sibling, daycare	9 High
Ong 2014	Retrospective	62576	0-1year	3-7 years	Database	OR 1.05(0.90-1.10)	Yes	Yes	Gender, residence	8 High
Metsala 2014	Prospective	6690	0-1 year	3-9 years	Database	OR 1.46(1.30-1.64)	Yes	Yes	Gender, district, maternal asthma, gestational age	8 High
Hoskin 2013	Prospective	4952	0-2 years	7.5 years	Questionnaires	OR 1.09(0.84-1.41)	Yes	No	Gender, ethnicity, gestational age, delivery mode, birth weight, breastfeeding, maternal education, pets, maternal antibiotics use, smoking	7 High
Wang 2013	Prospective	263620	0-1 year	2-6 years	Database	HR† 1.38(1.32-1.46) 0.96(0.72-1.29)	Yes	Yes	Healthcare utilization, gender, paracetamol, residence	8High 9High†
Moraes 2013	Cross-sectional	1060	0-1 year	1.5 years	Questionnaires	OR 2.29(1.18-4.46)	No	Yes	Sex, ethnicity, mother's age, birth mode, birthweight, socioeconomic status, educational breastfeeding, time spent outdoors, disinfectant use by mother during pregnancy, mother's smoking during pregnancy, pets	7 Medium
Muc 2013	Cross-sectional	1063	0-1 year	5-9 years	Questionnaires	OR 1.60(1.03-2.48)	No	No	Age, gender, preterm, rhinitis	4 Medium
Goksor 2013	Prospective	4051	0-1 week	8 years	Questionnaires	OR 2.30(1.20-4.20)	No	No	Gender, familial allergy, gestational age, delivery mode, birth weight, breastfeeding, smoking, maternal education, outdoors activity	6 Medium
Van 2013	Prospective	1042	0-1 year	7-11 years	Questionnaires	OR 1.66(0.89-3.11)	Yes	No	Familial allergy, residence	6 Medium
Almqvist 2012	Prospective	211192	0-1 year	3 years	Database	HR 0.71(0.41-1.23)	Yes	Yes	Not provided	7 High

Table S1 (Continued)

Study	Study design	Sample size	Exposure timing	Asthma age	Measurements	Effect size, 95%CI	RC	CbI	Adjusted model	Quality score
Kwon 2011	Cross-sectional	5036	0-1 year	7-16 years	Questionnaires	OR 1.88(1.35-2.62)	No	No	Age, sex, BMI, parental asthma, degree of maternal education, secondhand smoking	6 Medium
Risnes 2011	Prospective	1401	0-6 months	6 years	Questionnaires	OR 1.66(0.99-2.79)	Yes	Yes	Outcome, parental asthma	6 Medium
Rusconi 2011	Cross-sectional	16933	0-1 year	6-7 years	Questionnaires	OR 1.18(1.02-1.38)	Yes	Yes	Age, parental schooling, questionnaire's completion season, the person who completed the questionnaire, parental atopy, maternal smoking, sex, prematurity, maternal age, breastfeeding, siblings, day care, sleep in the same bedroom with other children, damp in child's bedroom	9 High

Abbreviations: OR, odds ratio; RR, relative risk; HR, hazard ratio; CI, confidence interval; RC, reverse causation; CbI, confounding by indication; BMI, body mass index; NICU, neonatal intensive care unit.

†: Records from two different studies, as two separate studies were included in the same article.

‡: For cohort studies and case-control studies, score of 4-6 was considered as medium quality and 7-9 as high quality. For cross-sectional studies, score of 4-7 was considered as medium quality and 8-11 as high quality.

Table S1 (continued) Characteristics of included studies in the meta-analysis

Study	Region	Race, n (%)	District, n (%)	Asthma diagnosis	Antibiotic type, n (%)	Antibiotic duration	No. of cases, percentage(%)
Aversa 2021	US	White: 10348 (71%) Black: 1299 (8.9%) Asian: 955 (6.6%) Hawaiian: 52 (0.4%) American Indian: 46 (0.3%)		According to the International Classification of Disease, Ninth Revision, Clinical Modification	Penicillins: 9306 (54.1%) Cephalosporins: 3401 (19.8%) Sulfonamides: 777 (4.5%) Macrolides: 3724 (21.6%)	Electronic prescriptions were retrieved from the proprietary electronic systems of each institution	1176, 8.2%
Zou 2020	China	Asian: 13335 (100%)	Urban: 9014 (67.6) Suburban: 4062 (30.5)	A positive answer to the written question: "Have you (has your child) had wheezing or whistling in the chest in the past 12 months?"		Parents/guardians provided information regarding first-year antibiotics exposure via the question "Had your child been treated by antibiotics during the first year of the child's lifetime (yes vs. no)"	1285, 9.6%
Patrick 2020	Canada		Rural: 8,938 (25.6%) Rural: 2,385(30.2%)†	Consistent and typical clinical history of symptoms and objective evidence of bronchodilator response, with ≥ 12% change in Forced Expiratory Volume in 1 sec			118, 6.1%
Slob 2020	Netherlands Sweden			International Classification of Disease; Questionnaire and two or more prescriptions for preventer medications or 3 SABA prescriptions or 2 SABA and 1 preventer medication.	Narrow spectrum: 3201 (70.0%) Broad spectrum: 1381 (30.0%)	Exposure to antibiotics was defined between 0-2 years of age as any parental-reported antibiotics use in Dutch twins and as any	5181, 15.1% 1381, 17.5%†

						prescription claim for antibiotics (ATC codes) in the SPDR for Swedish twins	
Lin 2020	Taiwan, China	Asian: 3236(100%)	Urban 3516 (27.2%) Suburban 4809 (37.1%) Rural 4619 (35.7%)	Patients who were diagnosed with asthma had at least two prescriptions of anti-asthmatic drugs at different times within a 1-year period. Anti-asthmatic drugs included inhaled selective β2-agonists, inhaled corticosteroids, combined inhaled salbutamol/sodium cromoglycate, and combined inhaled selective β2-agonists/corticosteroids.	Penicillins: 7178 (32.5%) Cephalosporins: 10351 (46.8%) Macrolides: 4567 (20.7%)	Antibiotic exposure was defined as at least one prescription of antibiotic (ATC code J01) within 5 years preceding the date of asthma diagnosis	
Donovan 2020	US	White: 84 053 (55.1%) Black: 55 862 (36.6%)	Urban: 52 162 (34.2) Suburban: 35 616 (23.3) Rural: 64 713 (42.4)	Asthma was ascertained between ages 4.5 years and 6 years using asthma-related healthcare encounters and asthma-specific medication use.		Antibiotic use during the first 12 months of life was captured from medical claims prescription fill data of TennCare	20984, 14.0%
Fishman 2020	US		Urban or suburban: 66526(83.5%) Rural: 13169(16.5%)	Asthma was identified by at least 1 inpatient diagnosis (ICD-9 code beginning 493 or ICD-10 code beginning J45), at least 2 outpatient or emergency department diagnoses, or at least 2 prescription fills for asthma drugs		The main exposure was the number of prescriptions filled for systemic antibiotics,31 identified by National Drug Codes in paid pharmacy claims	2381, 3.0%
Neto 2020	Brazil			Current asthma was indicated based on an affirmative answer to the question “Has your child had wheezing or whistling in the chest in the past 12 months?”			339, 38.6%
Morata 2019	Spain	Caucasian: 103 (88.8%)	Urban: 93 (80.2%)	Asthma is defined as persistent or recurrent broncho-obstructive symptoms (cough, wheezing			88, 38.0%

		Gypsy: 5 (4.3%) Arabic: 4 (3.4%) Chinese/other: 4 (3.5%)	Rural: 23 (19.8%)	and/or dyspnoea) with a good response to bronchodilators, along with an affirmative answer to the ISAAC questionnaire question: “Have you had any wheezing in the past 12 months?”		
Toivonen 2019	Finland	Finnish: 923 (100%)		Asthma diagnoses and medications were retrieved from medical records and electronic prescriptions at age 7 years		63, 8.1%
Ni 2019	US	White: 909 (37.9%) Black: 499 (20.8%) Hispanic: 760 (31.7%)		According to the International Classification of Disease, Ninth Revision, Clinical Modification	Oral or intravenous antibiotic records	266, 11.1%
Arrais 2019	Angola			Positive replies to the question “Has your child had wheezing or whistling in the chest in the past 12 months?”	Questions on use of antibiotics in their child's first year of life	739, 24.0%
Norback 2019	China	Asian: 39782 (100%)	Urban: 29262(74.0%) Suburban: 7786 (20.0%) Rural: 1520 (4.0%)	One yes/no questions asking if the child had ever been diagnosed by a doctor for asthma	One question asked if the child had ever used antibiotics, with three alternatives: never, first year of life, and after first year of life	2936, 7.4%

Metzler 2019	Austria, Finland, France, Germany, Switzerland			Asthma was defined as doctor diagnosis or at least two doctor-diagnosed episodes of obstructive bronchitis in the last 12 months in the year 4, 5 or 6		Information on antibiotic exposure during the first year of life including information on indication was obtained from the 2-month and 12-month questionnaires.	79, 8.9%
Silverwood 2019	UK			A positive response to the question “Has your child/have you had wheezing or whistling in the chest in the past 12 months?”			38219, 10.3%
Soto 2019	Costa Rica			A positive answer to the written question: “Have you (has your child) had wheezing or whistling in the chest in the past 12 months?”			663, 23.5%
Chen 2018	Taiwan, China	Asian: 4164 (100%)	Urban: 914(21.9%) Suburban: 1624 (39.0%) Rural: 1626 (39.0%)	According to the International Classification of Disease, Ninth Revision, Clinical Modification	Penicillins: 2761 (50.5%) Cephalosporins: 1410 (25.8%) Macrolides: 1296 (23.7%)	Antibiotic use was defined as the prescription of at least one antibiotic (anatomical therapeutic chemical [ATC] code J01*) in the 5 years before onset of asthma	

Mitre 2018	US	According to the International Classification of Diseases, Ninth Revision, Clinical Modification		The MHS database was used to identify children prescribed an outpatient antibiotic at any time prior to age 6 months using the American Hospital Formulary Service classification	111792, 14.1%
Yoshida 2018	Japan	According to ICD J45 and administered ICS and other controllers as asthma medication, such as leukotriene-receptor antagonists, long-acting beta 2 agonists, short-acting beta 2 agonists, theophylline and disodium cromoglycate	Penicillins: 16992 (24.5%) Cephalosporins: 31314 (42.2%) Macrolides: 20881 (30.2%) Penicillins: 5666 (24.1%) Cephalosporins: 10701 (45.5%) Macrolides: 7132 (30.3%)†	Infant exposure was defined as antibiotics prescribed during the first year after birth and information on prescription of antibiotics (ATC J01) was collected	3363, 4.4% 1582, 5.6%†
Stromberg 2018	Sweden	Current asthma at 12 years of age was defined as reported doctor-diagnosed asthma and either current treatment or symptoms.			233, 6.4%

Oosterloo 2018	Netherlands			Wheezing was defined as a whistling, wheezy sound coming from the lower airways during expiration, but not when coming from or through the nose		A monthly questionnaire was filled out concerning antibiotic treatment	149, 34.2%
Yamamoto 2017	Japan	Asian: 1196 (100%)		A positive answer from the caregiver to the question (child at 5 y old), "Has your child ever been diagnosed by a doctor as having asthma in the past 12 months?"	Penicillins: 73 (16.6%) Cephalosporins: 194 (44.1%) Macrolides: 173 (39.3%)	According to a positive answer from caregivers to the question (child at 2 y old), "Has your child ever taken antibiotics?"	95, 10.5%
Ahamadizar 2017	Netherlands UK			A positive response to the following questions at age of 9- 10 years in Generation R and at age of 10 years in SEATON "has your child ever suffered from asthma?" and "was this confirmed by a doctor?"		Antibiotic use in the Generation R cohort (first 3 years) and in the SEATON cohort (first 6 months) was available from parental-reported	448, 9.5% 74, 8.3%†
Han 2017	US Argentina		Urban: 1121 (73.9%) Rural: 396 (26.1%)	A positive answer to the written question: "Have you (has your child) had wheezing or whistling in the chest in the past 12 months?"		Information on any use of antibiotics in the first year of life was obtained from the core questionnaire	58, 3.8%
Gupta 2016	US	White: 1012 (74.5%) Black: 72 (5.3%) Hispanic: 141 (10.4%) Asian: 35 (2.6%)		A physician diagnosis was required as well as the age of diagnosis. Asthma medications were also reported.		For antibiotic use, the parents were asked about the number of times that their child took antibiotics in his or her first year of life	406, 30.0%
Wu	US	White:		Using the International Classification of		Data of antibiotic was	18018, 13.2%

2016		64830 (50.1%) Black: 48759 (37.7%) Hispanic: 7321 (5.7%) Asian: 8410 (6.5%)	Diseases, Ninth Revision, Clinical Modification and was ascertained using healthcare encounters and pharmacy claims		retrieved from medical claims prescription fill data	
Pitter 2016	Italy		A subject had at least two prescriptions, at different time points within a 12-month period, of any of the following anti-asthmatic drugs: inhaled selective β2-agonists; inhaled corticosteroids; combined inhaled salbutamol/sodium cromoglycate; combined inhaled selective β2-agonists/corticosteroids	Penicillins: 11756 (55.8%) Cephalosporins: 5207 (24.7%) Macrolides: 4114 (19.5%)	The prescription of at least one antibiotic (ATC code J01*) during the first 12 months of life	34957, 24.4%
Krenz 2015	Poland		Children with doctor-diagnosed cases and the ones with positive answers to all questions in module 1.1 of the ISAAC protocol			130, 14.5%
Lee 2015	Korea	Asian: 7389 (100%)	The asthma diagnosis was determined by the question "Have you ever been diagnosed with asthma?".		Information on antibiotic exposure in the first year of life was determined using a questionnaire.	908, 14.3%
Eldeirawi 2015	US		Doctor-diagnosed asthma were derived from the question, “Has a doctor ever said that your child had asthma?”		Antibiotic use in infancy was assessed by the question: ‘‘In his/her first year of life, did your child receive antibiotics (including not prescribed) to treat an infection?’’	707, 7.4%
Sun 2015	England Germany Austria		By the question “Has your child had wheezing or whistling in the chest in the last 12 months?” at 6, 12, 24 and 36 months of age	Ampicilline: 47 (32.6%) Penicillins: 15 (10.4%) Cephalosporins:	An annual antibiotic use diary was provided to the parents for each year of study	29, 4.7%

				50 (34.7%) Macrolides: 29 (20.1%) Trimethroprim: 3 (2.1%)	
Ortqvist 2014	Sweden		A diagnosis using the international classification of diseases, 10th revision and fulfilled one or both of two criteria for asthma drugs: two or more dispensed inhaled corticosteroids, leukotriene receptor antagonists, or fixed combinations of β2 agonists and corticosteroids, with gaps of two or more weeks between distributions or three or more dispensed asthma drugs as previously mentioned or short acting β2 agonists within a year	Information on generic names and dates of filled prescriptions of systemic antibiotics (Anatomical Therapeutic Chemical (ATC) classifications system code J01A-J01X) was retrieved	29753, 6.0% 11260, 6.2%†
Khalkhali 2014	Iran	Urban: 374 (92.8%) Rural: 29 (7.2%)	To recruit incident asthmatic patients, we adopted the Global Initiative for Asthma criteria	Data related to the history of antibiotic consumption in the first year of life and during pregnancy were collected by interviewing the parents/guardians	

Lapin 2014	US			Asthma was defined as ever having an asthma diagnosis by 3 years of age.		Early antibiotic use, excluding topical antibiotics or antifungal agents, was determined at 5 time points during the first year of life	44, 15.0%
Semic 2014	UK			Wheezing confirmed by the child's general practitioner; age (in days) of the first episode was recorded (medical records)		Age (in days) when the child was prescribed the first antibiotic (medical records)	409, 51.0%
Ong 2014	US		Urban: 49897 (79.7%) Rural: 12679 (20.3%)	Children with asthma were identified using a revised definition of the Health Employers Data Information System criteria for the National Committee on Quality Assurance definition and was diagnosed using ICD-9 codes		The receipt of at least one prescription of antibiotic in the first year of life. One course of antibiotic prescription was defined as a single continuous prescription, typically lasting 7 to 10 days	11576, 18.5%
Metsala 2014	Finland			Information on diagnosed asthma was obtained from the Special Reimbursement Register.	Penicillins: 1491 (38.3%) Cephalosporins: 1322 (34.0%) Macrolides: 539 (13.8%)	Information on antibiotics was obtained from the Drug Prescription Register maintained since 1994.	
Hoskin 2013	UK			The primary outcome was asthma at approximately 7.5 yr defined as maternal report of a physician's diagnosis of asthma at any time and symptoms of wheezing during the previous 12 months		Mothers reported whether their child had taken antibiotics (‘No’, ‘Yes, once’ or ‘Yes, twice or more’) in periods 0–6, 6–15 and 15–24 months	630, 10.9%
Wang 2013	Taiwan, China	Asian: 263620 (100%)	Urban: 72 925(27.66%) Suburban: 160847(61.01%)	At least one inpatient claim record for asthma (A code: A323 or ICD-9 code = 493), or two ambulatory claims coded as A code = A323 or ICD-9 code = 493		Prescription records regarding antibiotic use occurring within the windows 1998–99 and	57328, 21.7%

		Rural: 24 829 (9.42%) Urban: 2921 (29.48%) Suburban: 5806 (58.59%) Rural: 900 (9.08%)†			2003–04, respectively, were extracted according to Anatomic Therapeutic Chemical Classification System (ATC) codes	
Moraes 2013	Latin American countries; Spain; Netherlands		Infants who had three or more episodes of wheezing were deemed recurrent wheezers			294, 27.7%
Muc 2013	Portugal		A positive response to a question of a child ever having an asthma attack		According to answers to the question: “In the first 12 months of life, did your child have any antibiotics?”	106, 10.4%
Goksor 2013	Sweden		The diagnosis of current asthma at 8 years of age was based on the answers to Q1: “Has your child been diagnosed with asthma by a physician?” Q2: “Has your child received medication for asthma during the last 12 months?” Q3: “Has your child had problems with/symptoms of wheezing during the last 12 months?”			167, 5.7%
Van 2013	Cuba	Urban: 449 (56.1%)	Asthma was defined as an affirmative answer to the second ISAAC core asthma question on current wheeze (“Has your child had wheezing or whistling in the chest in the last 12 months?”)		Antibiotic use during the child’s first year of life was assessed by questionnaire	31, 3.9%
Almqvist	Sweden		Asthma was defined as any asthma	Amoxicillin:	Aispensed antibiotics	28492, 13.5%

2012			medication, except oral β_2-agonists, dispensed at least twice	84607 (27.8%) Penicillins: 170120 (55.9%) Cephalosporins: 26299 (8.6%) Macrolides: 23509 (7.7%)	(ATC-codes J01) at some point between birth and 31 July 2009	
Kwon 2011	Korea		Current asthma was defined when children had been diagnosed with asthma in their lifetime and experienced wheezing within 12 months of the survey			343, 7.9%
Risnes 2011	US	White: 1032 (73.7%) African American: 134 (9.6%) Hispanic: 178 (12.7%) Asian/other: 57 (4.1%)	Physician-diagnosed asthma after 6 months of age with history of wheezing in the sixth year of life.		When the child was 6 years (±3 months) of age, the mother was asked about "Has your child ever taken antibiotics?"	66, 4.7%
Rusconi 2011	Italy		Those who had at least one lower respiratory tract illness with wheezing in the first 2 years of life, and had no wheezing in the last 12 months		Questions related to antibiotics were: "In the first 12 months of your child's life, did you give antibiotics?"	1011, 6.0%

Abbreviations: OR, odds ratio; RR, relative risk; HR, hazard ratio; CI, confidence interval; RC, reverse causation; CbI, confounding by indication; BMI, body mass index; NICU, neonatal intensive care unit; ICD, the international Classification of Disease; ATC, Anatomic Therapeutic Chemical Classification System.

†: Records from two different studies, as two separate studies were included in the same article.

Quality assessment							Effect	
No of studies	**Design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**	**Relative (95% CI)**	**Quality**
23	observational studies	no serious risk of bias	serious[1]	no serious indirectness	no serious imprecision	dose response gradient[2]	OR 1.19 (1.11 to 1.28)	⊕⊕OO LOW

Table S2 Summary of GRADE criteria

[1] High heterogeneity was observed among included studies.

[2] Studies providing information on antibiotic course showed a dose-response relationship between infant antibiotic exposure and risk of childhood asthma.