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Maternal antibiotic exposure during pregnancy and the risk of allergic diseases in childhood: A meta-analysis

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The authors declare that they have no competing interest.

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Abstract

Background: Increasing studies suggest that antibiotic exposure during pregnancy may increase the risk of childhood allergic diseases; however, controversy still exists. Thus, we conducted this meta-analysis to evaluate the association between antibiotic use during pregnancy and childhood asthma/wheeze, eczema/atopic dermatitis and food allergy.

Methods: CENTRAL, EMBASE, and PubMed were searched for studies up to July 20, 2020. Cohort studies and case-control studies that estimated the association of antibiotic exposure in pregnancy with the risk of childhood asthma/wheeze, eczema/atopic dermatitis and food allergy were included. A random-effects model or fixed-effects model was used to calculate the pooled estimates. The quality of the included studies was assessed by the Newcastle-Ottawa Scale (NOS). Stata12.0 software was used to analyze the association through a meta-analysis.

Results: A total of 26 studies were included in the meta-analysis. The results showed that maternal antibiotic exposure in pregnancy and the summary OR for the risk of childhood asthma/wheeze was 1.29 (95% CI = 1.16-1.43), the summary OR for eczema/atopic dermatitis was 1.62 (95%CI = 1.16-2.27), and the pooled OR for food allergy was 1.36 (95%CI = 0.94-1.96).

Conclusions: Our results indicated that maternal antibiotic use during pregnancy might increase the risk of asthma/wheeze and eczema/atopic dermatitis but not food allergy in children. Further studies with larger sample size and robust multivariable adjustment are needed to confirm our findings. Nevertheless, the appropriate use of antibiotics during pregnancy is incredibly important, and health care professionals should be selective when prescribing antibiotics for pregnant

women.

Key messages:

Our focus was narrowed on three common allergic diseases occurring during childhood: asthma/wheeze, eczema/atopic dermatitis and food allergy. Antibiotic use in pregnancy may increase the risk of asthma/wheeze and eczema/atopic dermatitis. Therefore, appropriate use of antibiotics during pregnancy is incredibly important, and health care professionals should prescribe antibiotics for pregnant women with caution in order to reduce the risk of allergic diseases in children.

Keywords: Pregnancy; antibiotic; childhood; asthma; wheeze; food allergy; eczema; atopic dermatitis; meta-analysis

Introduction

With the changes in lifestyle and health care system, antibiotics are being used on a large scale around the world¹. Due to the special physiology of pregnant women, they are more susceptible to infection, such as upper respiratory tract infection and urinary tract infection. Therefore, antibiotics are often used during pregnancy². It is estimated that more than 40% of pregnant women take antibiotics before delivery³. Meanwhile, as common allergic diseases among children, the worldwide prevalence of asthma/wheeze, eczema/atopic dermatitis and food allergy has increased evidently in the past decades⁴⁻⁶. These allergic diseases not only seriously affect the quality of patients' life, but also cause a huge personal and social-economic burden. It is reported that about \$56 billion per year is spent on asthma in the United States⁷.

The high frequency of antibiotic use in pregnant women and the prevalence of allergic diseases in children led to speculation about a cause-and-effect relationship. Some studies reported that maternal antibiotic exposure during pregnancy was associated with an altered infant gut microbiome composition^{8,9} which may lead to subsequent allergy diseases and obesity in childhood. It should be noted that much evidence suggested postnatal exposure to antibiotics might increase the risk of the development of allergic diseases in children¹⁰⁻¹⁷. For instance, Ahmadizar et al.¹⁰ found a positive association between early-life antibiotic exposure and the risk of developing symptoms such as eczema, hay fever and food allergy later in life through meta-analyses. Although an increasing number of studies have investigated the association between antibiotic exposure during pregnancy and the risk of development of allergic disease in childhood¹⁸⁻⁴³, the results were still inconsistent. Some studies reported that the correlation of antibiotic exposure during pregnancy and allergic disorders in children was significantly positive, while others did not observe any association. Two meta-analyses were found^{44,45}; however, both of them only focused on the association between antibiotic exposure during pregnancy and the risk of childhood asthma/wheeze; several latest pieces of research were missing as well.

The objective of this systematic review and meta-analysis was to further explore the association of antibiotic exposure during pregnancy with childhood asthma/wheeze and assess whether prenatal antibiotic exposure may also increase the risk of developing food allergy and eczema/atopic

dermatitis in subsequent children.

Methods

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guidelines⁴⁶.

Search strategy

A comprehensive literature search was conducted with Cochrane Central Register of Controlled Trials (CENTRAL), EMBASE, and PubMed databases up to July 20, 2020, to identify studies on the association between antibiotic exposure during pregnancy and childhood asthma or wheeze, eczema or atopic dermatitis and food allergy. Supplementary Table 1 showed a summary of detailed search strings for each database.

Eligibility criteria

Studies were included if they met the following inclusion criteria: (a) cohort studies or case-control studies published as original articles; (b) the exposure of interest was antibiotic use during pregnancy; (c) the outcome of interest was childhood asthma, wheeze, eczema, atopic dermatitis or food allergy; (d) studies that included raw data or reported the odds ratios (ORs), risk ratios (RRs) or hazard ratios (HRs) with their 95% confidence intervals (CIs) for the association between antibiotic use during pregnancy and the outcomes of interest; and (e) studies were published in English.

Studies selection and data extraction

After removing the duplicate references, the study titles and abstracts were screened by two independent investigators (Y Zhong and Y Zhang) to make sure they were relevant. Then, the studies that satisfied the eligibility criteria were included through full-text assessment. Subsequently, the two investigators collected the following data from each included study: first author's last name, year of publication, study design, country, population size, child's age,

exposure assessments, outcome assessments, the ORs/RRs/HRs with their 95% CIs and adjusted covariates. Moreover, if the outcomes at different ages were provided in the studies, the estimates of the longest follow-up period or the oldest age group were selected. If a study reported outcomes including asthma and wheeze simultaneously, the former estimates were selected in the meta-analysis. Discordant data abstraction was resolved by consensus and discussed with a third investigator (R Huang).

Quality assessment

The methodological quality of the included studies was assessed using the Newcastle-Ottawa Scale (NOS)⁴⁷. It consists of three parts, which are selection (0-4 points), comparability (0-2 points) and exposure/outcomes (0-3 points). Studies with scores of 0-3, 4-6 and 7-9 were considered to be of low, medium and high quality, respectively.

Statistical analysis

The adjusted ORs, RRs or HRs and their 95% CIs extracted from studies were used in the meta-analysis. If a study reported multiple estimates (different doses, trimesters or types of prenatal antibiotic use) without providing a total estimate of antibiotic use and an allergic disease of interest, the combined effect estimates were calculated first and then pooled into the meta-analysis. In our analyses, we pooled ORs, RRs, and HRs respectively if the effect estimates (ORs/HRs/RRs) differed across the included studies on a certain allergic disease. Heterogeneity across studies was assessed using the Q test ($P < 0.1$ was considered to have significant heterogeneity) and I^2 statistic ($I^2 > 50\%$ signified significant heterogeneity)⁴⁸. In the presence of significant heterogeneity, a random-effects model was adopted to pool the estimates across studies; otherwise, the fixed-effects model was selected. Subgroup analyses were conducted to explore the impact of various factors on the result of the association between antibiotic exposure and allergic disease in children. The robustness of our findings and potential sources of heterogeneity were checked by sensitivity analysis using 'one-study removed' method. To evaluate publication bias, Begg's funnel plot and Egger's test were applied^{49,50}. P -value < 0.05

was considered as statistical significance. All statistical analyses were conducted with Stata version 12.0 (Stata Corp, College Station, TX, USA).

Results

Study selection and characteristics

Initially, 2454 studies were identified based on our search strategies. Once the duplicates ($n = 306$) and the ineligible records ($n = 2099$) were removed by screening studies titles and abstracts, 49 full-text articles were retrieved for further assessment. In total, 26 studies were included in the final meta-analysis by the methods of study selection (Figure 1). Of the included studies, 20 were cohort studies^{18,20-23,25,26,28-31,34,36-43} and 6 were case-control studies^{19,24,27,32,33,35}. Twenty-one studies reported the association between maternal antibiotic use during pregnancy and the risk of developing childhood asthma or wheeze^{18-21,23-28,30,51}; eight articles explored the risk of eczema or atopic dermatitis in childhood^{21,22,25,29,31,34,38,41}; three studies investigated the correlation of antibiotic exposure with the incidence of food allergy in subsequent children^{22,32,34}. Concerning the study region, 17 studies were from Europe^{18,19,21,23,25,26,31-41}, 4 studies were from North America^{20,28,30,42}, and 5 were from Asia^{22,24,27,29,43}. Table 1 summarized the detailed characteristics of the included studies. Furthermore, the results of the quality assessment indicated that most of the included studies were of high quality, and only three studies were considered to be of medium quality (Supplementary Table2).

Analyses of antibiotic exposure during pregnancy and the risk of asthma/wheeze

13 out of 21 studies on childhood asthma/wheeze provided adjusted ORs (8 cohort studies^{18,20,21,25,26,28,34,42} and 5 case-control studies^{19,24,27,33,35}). The overall results of them indicated a statistically significant risk of developing childhood asthma/wheeze after maternal antibiotic use during pregnancy ($OR = 1.29$, 95% $CI = 1.16-1.43$, $I^2 = 92.2\%$, Figure 2). Similarly, the summary estimates of the studies which presented adjusted HRs (all cohort studies, $n = 5$)^{30,31,36,39,43} and adjusted RRs (all cohort studies, $n = 3$)^{23,37,40} also showed that prenatal antibiotic use may increase the risk of asthma/wheeze in subsequent children ($HR = 1.24$, 95% $CI = 1.19-1.30$, $I^2 = 62.6\%$,

Figure 3a; RR = 1.21, 95% CI = 1.17-1.24, $I^2 = 0$, Figure 3b, respectively). Supporting information contained the results of sensitivity analysis (Supplementary Figure 1a) and publication bias evaluation (Supplementary Figure 2).

Subgroup analysis: The pooled results of the association of antibiotic exposure with the risk of childhood asthma/wheeze for different characteristics of the included studies were shown in Supplementary Table 3. In the subgroup analysis based on study design, the results were as follows: studies from Europe (OR = 1.28, 95% CI = 1.14-1.43, $I^2 = 75.3\%$), studies from USA (OR = 1.18, 95% CI = 0.99-1.41, $I^2 = 97.0\%$) and studies from Asia (OR = 2.21, 95% CI = 1.20-4.05, $I^2 = 45.4\%$). For the subgroup analysis based on child's age, the association between antibiotic exposure and the risk of childhood asthma/wheeze was not significant in the subset of child's age < 3 (OR 1.75, 95% CI = 0.66-4.64, $I^2 = 84.3\%$). For the correlations between different types of antibiotic exposure and childhood asthma or wheeze, the correlation of macrolides with the risk of developing childhood asthma or wheeze was statistically significant (OR = 1.30, 95% CI = 1.11-1.52, $I^2 = 0$) but not for penicillins (OR = 1.23, 95% CI = 0.98-1.55, $I^2 = 95.8\%$), sulfonamides and trimethoprim (OR = 1.23, 95% CI = 0.87 -1.71, $I^2 = 82.9\%$), tetracyclines (OR = 1.09, 95% CI = 0.90-1.33, $I^2 = 36.3\%$) and nitrofurantoin (OR = 1.05, 95% CI = 0.95 -1.17, $I^2 = 0$). In terms of antibiotic exposure during different stages of pregnancy, all the summary ORs were positive but the highest OR was from the group of third trimester (OR = 1.17, 95% CI = 1.01-1.35, $I^2 = 91.9\%$). The results of the remaining subgroup analyses on the basis of study design, exposure assessment, sample size, year of publication, mother's age and adjusted covariates were identical with the overall results.

Analyses of antibiotic exposure and the risk of eczema/atopic dermatitis

Except for the study by McKeever et al.³¹, all the included studies on eczema/atopic dermatitis used the ORs to show the results^{21,22,25,29,34,38,41}. As for the overall meta-analysis of the seven studies with ORs, antibiotic use during pregnancy was significantly associated with the risk of developing eczema/atopic dermatitis in childhood (OR = 1.62, 95% CI = 1.16-2.27, $I^2 = 68.4\%$, Figure 4). The results of the sensitivity analysis were shown in Supplementary Figure 1b. We did

not evaluate publication bias since it was advised to analyze at least ten studies⁵².

Subgroup analysis: The results of subgroup analyses based on study design, exposure assessment, sample size, year of publication and adjusted covariates were shown in Supplementary Table 4. The results of the study subsets of using database or records, adjusting for delivery mode, sample size ≥ 1000 , year of publication ≥ 2015 , and mother's age ≥ 29 indicated that antibiotic exposure during pregnancy was not significantly associated with the risk of eczema or atopic dermatitis in children, while the association showed significance in the matched subgroups. For the subgroup analysis on the basis of study region, there was a stronger association if the studies were from Asia (OR = 3.18, 95% CI = 1.40-7.22, $I^2 = 0$) compared to those from Europe (OR = 1.46, 95% CI = 1.06-2.01, $I^2 = 68.8\%$).

Analyses of antibiotic exposure and the risk of food allergy

All three studies focusing on the association between antibiotic exposure during pregnancy and food allergy in subsequent children provided ORs as the effect estimates. Our meta-analysis revealed the statistical insignificance of the relationship (OR = 1.36 95% CI = 0.94-1.96, $I^2 = 29.4\%$, Figure 5). In addition, the subgroup analysis, sensitivity analysis and publication bias evaluation were not conducted due to the limited number of studies.

Discussion

To our knowledge, this meta-analysis was the first to assess the association between antibiotic exposure during pregnancy and multiple allergic diseases in subsequent children, food allergy and eczema/atopic dermatitis in particular. The results of our meta-analysis indicated that maternal antibiotic use in pregnancy was positively associated with an increased risk of childhood asthma/wheeze and eczema/atopic dermatitis. Our finding suggested that there was no statistically significant association between prenatal antibiotic use and food allergy in children. Moreover, only three studies were included, which meant that the overall risk estimate would be easily affected by individual studies. Hence, more researches are needed to assess the association between maternal antibiotic use during pregnancy and food allergy in childhood.

At present, the mechanism of the positive relationship between expectant mother's exposure to antibiotics and the risk of allergic disease in children is still unclear. Hygiene hypothesis indicates that microbial exposure in utero is crucial for the development of the immune system in children and the composition of the intestinal flora established early in life. A study found that the colonization of fetal microbes occurs as early as 11 weeks of gestation⁵³. Therefore, by inducing reduction and modification of the infant intestinal microbiota, early pregnancy antibiotic exposure may influence on immunological development, leading to an increase of the potential of chronic disease^{42,54}. In an animal study, maternal vancomycin treatment during pregnancy increased offspring allergic lung inflammation⁵⁵. Milliken et al.⁵⁶ reported that maternal use of antibiotics during the antenatal or labor could cause intestinal dysbiosis and particularly reduced diversity of *Lactobacillus* and *Bifidobacterium*. Similarly, Azad et al.⁵⁷ found a reduction of *Bacteroidetes* in 198 neonates whose mothers used antibiotics in labor. Some results of trials in mice revealed that antibiotics could promote the transition from T(H)1/T(H)2 balance to T(H)2 dominant immunity, while oral administration of intestinal flora could prevent the development of this process^{58,59}. Even so, this hypothesis is still controversial, and more clinical studies are needed to verify it in the future.

Previous articles have revealed that different types of antibiotics may affect the relationship between antibiotic use in pregnancy and allergic disease in subsequent children.^{18,20,30,32,33,35,42,43} A study reported that mere use of broad-spectrum antibiotics during pregnancy increased the risk of childhood asthma compared to an exposure limited to narrow-spectrum antibiotics⁴². Metsälä et al.³² found that the use of macrolide antibiotics in pregnant women also increases the risk of cow's milk allergy in children. In the present study, we selected five common antibiotics (penicillins, sulfonamides and trimethoprim, tetracyclines, nitrofurantoin and macrolides) for subgroup analysis to explore their impact on the risk of asthma/wheeze in children respectively, and the results showed that only macrolides were significantly associated with the risk of childhood asthma/wheeze.

As for the impact of different stages of pregnancy on childhood asthma/wheeze, a positive correlation between antibiotic exposure during each pregnancy trimester and the risk of childhood

asthma/wheeze was observed, and the finding was consistent with a previous meta-analysis⁴⁴. At the same time, we also noticed that there was a slightly higher risk in the third trimester than in the first or second trimester of pregnancy. Contrary to our finding, Bai et al.⁴⁴ reported there was a closer association between the use of antibiotics in the first trimester and the risk of asthma/wheeze in children. In addition, Timm et al.⁴¹ revealed that antibiotic use in the third trimesters of pregnancy did not increase the risk of atopic dermatitis in children. Therefore, more researches are needed to explore whether maternal antibiotic use at a particular trimester can produce different degrees of impact on the risk of children's allergic diseases. Some scholars have also noted there is a significant dose-response in the relationship between the risk of allergic diseases in children and antibiotic use during pregnancy. Wu et al.⁶⁰ reported a 13% increase in the risk of asthma in children with every additional course of antibiotics given to pregnant women. Similarly, Turi et al.⁴² found the relative odds of childhood asthma increased by 26% after an increase in prenatal antibiotic use.

Furthermore, antibiotic exposure during pregnancy and allergic diseases in subsequent children were influenced by many maternal characteristics such as maternal age, maternal history of allergy, maternal smoking, delivery mode and maternal educational level^{18,19,21,23,24,29,30,32,41,61}. Hence subgroup analysis was performed based on whether the study adjusted for these factors. For antibiotic exposure and asthma/wheeze in children, the pooled result of studies adjusted for maternal allergy was the same as the overall result. However, the summary result of studies without adjustment for maternal allergy showed statistical insignificance in the association between antibiotic use in pregnant women and asthma/wheeze in children. For prenatal antibiotic exposure and eczema/atopic dermatitis in children, after neglecting the subsets that included only one study, the remaining subsets of studies controlling for maternal allergy, maternal age, and mode of delivery showed different results from their counterparts. Therefore, maternal characteristics should be strictly controlled in future researches to obtain more stable and accurate results.

For the association between antibiotic use and childhood asthma/wheeze, we pooled the ORs, HRs and RRs, respectively. We found that results were consistent (they had nearly the same direction

and magnitude), indicating our conclusion, that prenatal exposure to antibiotics could increase the risk of wheeze or asthma in children, was robust and reliable. Sensitivity analyses proved the stability of our results as well (Supplementary Figure 1a, b). However, for antibiotic exposure during pregnancy and childhood eczema/atopic dermatitis, we observed that the study by Timm et al.⁴¹ contributed substantial heterogeneity. The reasons may be as follows: First, information about prenatal antibiotic exposure and atopic dermatitis was obtained from parents' reports during interviews in the study, which may increase the risk of misstatement and underreporting, weakening the association between antibiotic exposure and atopic dermatitis in children. Second, compared with other studies, this study only adjusted for a small number of covariates and hardly adjusted for critical confounding factors (e.g., infant gender, maternal allergy history, maternal age, etc.). Besides, for the association between antibiotic use and childhood asthma/wheeze, we also found that year of publication and the adjusted covariates (delivery mode and maternal education) may be the source of heterogeneity across the studies.

It is undeniable that several limitations in our meta-analysis yet exist. 1) For the associations between antibiotic exposure during pregnancy and eczema/atopic dermatitis and food allergy in children, the findings should be considered hypothetical and exploratory due to the limited number of included studies. Similarly, some subgroup analyses were also unconvincing since these subsets contained few studies (some contained only one or two studies). Hence the results of these subgroups need to be interpreted with caution. 2) The included study populations are mainly European, thus the conclusions may not be suitable for other countries and regions. Indeed, our pooled result of studies from the USA suggested that the association between antibiotic exposure in pregnancy and asthma/wheeze in children was not statistically significant. 3) The adjusted confounding factors of individual studies were inconsistent. Although most of the studies have adjusted for major confounding factors, such as mother's age, smoking during pregnancy, and maternal history of allergy, few studies adjusted for maternal body mass index (BMI), household socioeconomic, maternal use of other drugs and pets at home. In addition, children's characteristics, such as antibiotic use in early life and age, are also critical confounding factors. Some previous studies have reported that exposure to antibiotics in the first two years of life could

increase the risk of childhood asthma/wheeze¹³⁻¹⁷, eczema^{11,12,14} and food allergy³². Metzler et al.³⁴ found that the strongest effect of antibiotic exposure in utero was on atopic dermatitis and food allergy with onset during the first year of life. However, in our subgroup analysis, children older than three years of age were more likely to develop asthma/wheeze than those younger than three if the mother used antibiotic during pregnancy. 4) There was a publication bias in the analysis of the association between antibiotic use in pregnant women and asthma/wheeze in children (Supplementary Figure 2). This means that some articles with negative results have not been published yet. In addition, we did not search for master's or doctoral dissertations, grey literature, conference abstracts, letters to the editor, etc., which may cause some literature omission. We also noted that some study subsets (case-control study, smaller sample size, unadjusted maternal smoking and unadjusted maternal education) had significantly higher estimates than the corresponding study subsets. These low-quality studies may also be the cause of the publication bias.

Conclusion

In summary, this meta-analysis indicated that maternal antibiotic use during pregnancy was associated with an increased risk of asthma/wheeze and eczema/atopic dermatitis, but not with food allergy in children. In view of the limitations of the present study, more large-scale, well-designed prospective studies are needed to adequately adjust for multiple confounding factors, especially the important characteristics of mothers and children, to verify our results. Additional study is expected to further explore the mechanism of the association between antibiotic exposure during pregnancy and the risk of allergic disease in children. It is important that health care professionals should be selective when prescribing antibiotics for pregnant women to reduce unnecessary antibiotic use, and further prospective work is needed to guide the prescription of antibiotics during pregnancy.

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Impact statement

We conducted this meta-analysis to evaluate the association between antibiotic use during pregnancy and allergic disease in childhood. There are several strengths in our study. First, we simultaneously focused on three common allergic diseases in childhood, including asthma/wheeze, eczema/atopic dermatitis and food allergy. Second, some potential factors which may have an influence on the result were discussed in the manuscript. Third, we found antibiotic use in pregnancy may increase the risk of asthma/wheeze and eczema/atopic dermatitis, which indicates the appropriate use of antibiotics during pregnancy is particularly important.

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Figure legends

Figure 1. Flow chart of search process and reasons for exclusion.

Figure 2. Forest plot of antibiotic exposure during pregnancy and risk of childhood wheeze/asthma (overall meta-analysis of the studies provided adjusted ORs)

Figure 3. Forest plot of antibiotic exposure during pregnancy and risk of childhood wheeze/asthma (a: overall meta-analysis of the studies provided adjusted HRs; b: overall meta-analysis of the studies provided adjusted RRs)

Figure 4. Forest plot of the association between antibiotic exposure during pregnancy and risk of eczema/atopic dermatitis in children.

Figure 5. Forest plot of the association between antibiotic exposure during pregnancy and risk of food allergy in children.

Supporting information

Supplementary Table 1. Search strategies for each database

Supplementary Table 2. Assessment of quality of the studies according to the Newcastle-Ottawa

Scale (NOS)

Supplementary Table 3. Subgroup analyses of the association between maternal antibiotic use during pregnancy and the risk of childhood asthma/wheeze

Supplementary Table 4. Subgroup analyses of the association between maternal antibiotic use during pregnancy and the risk of childhood eczema/atopic dermatitis

Supplementary Figure 1. Sensitivity analysis of the included studies on the association between antibiotic exposure during pregnancy and allergic diseases in children (a: childhood asthma/wheeze; b: childhood eczema/atopic dermatitis)

Supplementary Figure 2. Begg's funnel plot of the included studies on the association between antibiotic exposure during pregnancy and childhood asthma/wheeze

Table 1. Characteristics of the included studies

Author (year)	Country	Sample size	Exposure measurement	Age	Outcome measurement	Estimates (95% CI)	Adjusted covariates
Cohort study							
Benn et al. ¹⁸ (2002)	Denmark	2742	Parent-report	4-5	Database for prescriptions	Asthma, OR: 1.80 (1.00-3.10)	Low gestational age, maternal age, maternal education, maternal allergic disease, and breast-feeding
Chu et al. ²⁰ (2015)	USA	39907	Parent-report	0-7	Medical records	Asthma, pooled OR: 1.03 (1.01-1.05)	Maternal age at delivery, marital status at pregnancy, race, educational level, parity, smoking during pregnancy, maternal asthma history, and maternal history of drug allergy
Dom et al. ²¹ (2010)	Belgium	773	Parent-report	0-4	Parent-report	Eczema, OR: 1.82 (1.14-2.92) Recurrent wheeze, OR: 1.29 (0.80-2.09)	Gender, maternal age, birth weight, siblings, breastfeeding, pre- and post-natal exposure to cats or dogs, pre- and post-natal exposure to cigarette smoke, day care attendance, parental education and parental history of allergies
Gao et al. ²² (2019)	China	903	Parent-report and telephone interviews	0-1	Assessed by a well-trained investigator at home visit and interview	Eczema, OR: 3.59 (1.19-10.85) Food allergy, OR: 1.28 (0.41-3.97)	Infant's sex, parental history of allergy, maternal age, maternal smoking, maternal education and caesarean section

Higgins et al. ²³ (2020)	UK	412	Parent-report and records of hospital and birth	0-1	Parent-report	Wheeze, RR: 1.01 (0.69-1.47)	Maternal asthma, smoking during pregnancy, maternal infection, and age
Jedrychowski et al. ²⁵ (2006)	Poland	102	Prenatal interview	0-1	A standardized interview	Eczema, OR: 2.30 (0.91-5.80) Wheeze, OR: 1.14 (1.01-1.27)	Maternal education, child's gender, and maternal allergy
Källén et al. ²⁶ (2013)	Sweden	12395	Database	≥ 2	Records of prescriptions	Asthma, OR: 1.10 (1.02-1.19)	Year of birth, maternal age, parity, smoking, and BMI
Lapin et al. ²⁸ (2015)	USA	295	Parent-report	0-3	Self-reported answers to the question	Asthma, OR: 3.12 (1.44-6.77)	Maternal asthma, any smoking in pregnancy, child antibiotic use, child exposed to smoke in the home.
Lee et al. ²⁹ (2014)	Korea	412	Medical records	0-1	Clinical diagnosis	Atopic dermatitis, OR: 2.73 (0.80-9.30)	Gestational age at birth, sex, pre-pregnancy maternal body-mass index, maternal age at delivery, maternal education level, prenatal exposure to smoke, prenatal exposure to pets, and presence of older sibling(s)
Loewen et al. ³⁰ (2018)	Canada	213418	Records of prescriptions	≥ 5	Hospitalization for asthma, physician diagnosis of	Asthma, HR: 1.23 (1.20-1.27)	Maternal asthma, sex, location of residence, length of gestation, number of children in household, and income quintile, and any infant

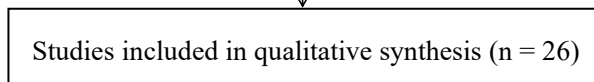
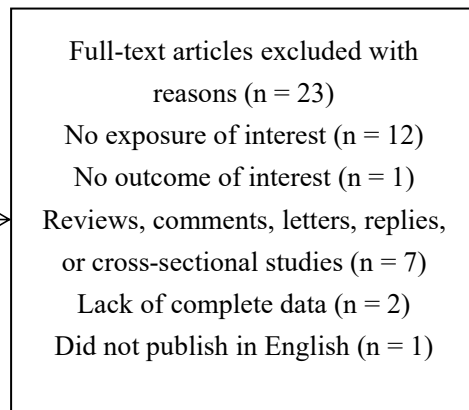
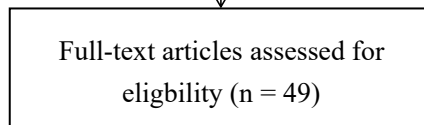
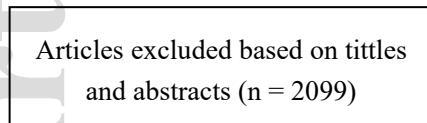
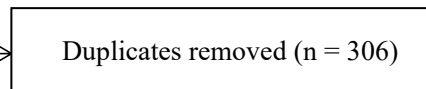
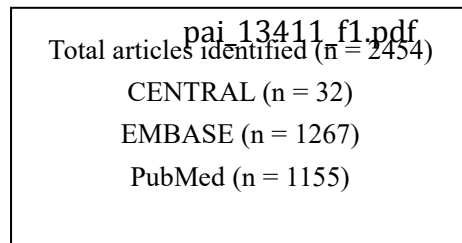
					asthma, and prescriptions		antibiotic use before 12 months of age
McKeever et al. ³¹ (2002)	UK	24690	Database for antibiotics prescriptions	0-10	Database	Asthma, pooled HR: 1.35 (1.13-1.62) Eczema, pooled HR: 1.04 (1.00-1.08)	Consulting behavior and maternal allergic disease
Metzler et al. ³⁴ (2019)	Switzerland	1080	Parent-report	0-6	Doctor diagnosis of allergic diseases obtained from questionnaires	Atopic dermatitis, OR: 1.23 (0.73-2.09) Asthma, OR: 1.36 (0.59-3.11) Food allergy, OR: 2.39 (1.08-5.26)	Farmer, centre, parental atopic status, gender, smoking during pregnancy, number of siblings, pets during pregnancy, caesarean section, and maternal education.
Örtqvist et al. ³⁶ (2014)	Sweden	493785	Records of Prescriptions	0-5.5	Doctor diagnosis of asthma registered in database	Asthma, HR: 1.28 (1.25-1.32)	Maternal smoking during pregnancy, parity, family situation, mother's country of birth, parental education, maternal asthma, and age as analysis time scale
Popovic et al. ³⁷ (2016)	Italy	7515	Parent-report	0-1.5	Parent-report	Recurrent wheeze, pooled RR: 1.48 (0.71-3.07)	Maternal educational level, maternal age at birth, smoking in pregnancy, siblings, paracetamol use in pregnancy and maternal asthma or asthmatic

							bronchitis, maternal respiratory infections, other maternal infections, and fever >38°C
Sariachvili et al. ³⁸ (2007)	Belgium	976	Parent-report	0-1	Parent-report	Eczema, OR: 1.80 (1.20-2.70)	Maternal atopy, maternal age, smoking during pregnancy, and maternal educational level
Stensballe et al. ³⁹ (2013)	Denmark	18606	Records of Prescriptions	0-5	Records of hospitalization	Asthma, HR: 1.32 (1.12-1.56)	Parental asthma, maternal age at delivery, type of birth, and pets
Stokholm et al. ⁴⁰ (2014)	Denmark	864689	Records of Prescriptions	0-14	Database	Asthma, pooled RR: 1.21 (1.17-1.24)	Age and calendar year, birthweight, gestational age, sex, mode of delivery, parity, multiple births, season of birth, maternal age, smoking during pregnancy, maternal employment status, and maternal asthma
Timm et al. ⁴¹ (2017)	Denmark	62560	Interviews	0-1.5	Parent-report at the interviews	Atopic dermatitis, Pooled OR: 1.07 (0.98-1.17)	Prenatal smoking, household socioeconomic, and older siblings
Turi et al. ⁴² (2020)	USA	84214	Medical records of prescriptions	4.5-6	Pharmacy claims and asthma related healthcare encounters	Asthma, OR: 1.23 (1.18-1.28)	Maternal age, asthma, education, smoking, presence of group B streptococcus infection, delivery method, infant characteristics including birth hospitalization

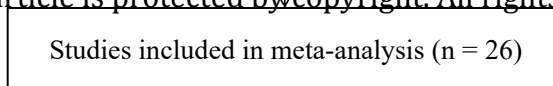
							length of stay, gestational age, birth weight, infant race, sex and number of living siblings at home
Yoshida et al. ⁴³ (2018)	Japan	41808	Records of prescription	3-6	Diagnosis of asthma with prescription and one or more control therapies	Asthma, HR: 1.09 (0.97-1.22)	Sex
Case-control study							
Calvani et al. ¹⁹ (2004)	Italy	805	A structured interview	≥ 3	Medical therapy or doctor diagnosis	Asthma, OR: 1.65 (0.92-2.93)	Age, sex, maternal asthma, maternal smoking during pregnancy, skin prick test positivity and atopic dermatitis
Huo et al. ²⁴ (2018)	China	1498	A face-to-face interview	3-12	Diagnosis of asthma by pediatricians	Asthma OR: 1.70 (1.00-2.90)	Maternal age at delivery, maternal education level, child age, child gender, gestational age at birth, delivery modes, family history of allergic disorders, breastfeeding within the first 6 months of life
Kashanian et al. ²⁷ (2017)	Iran	268	Records of hospitals	7-14	Diagnosis of asthma by two physicians	Asthma, OR: 3.19 (1.52-6.67)	Maternal asthma, maternal age, vaginal bleeding during pregnancy, breastfeeding

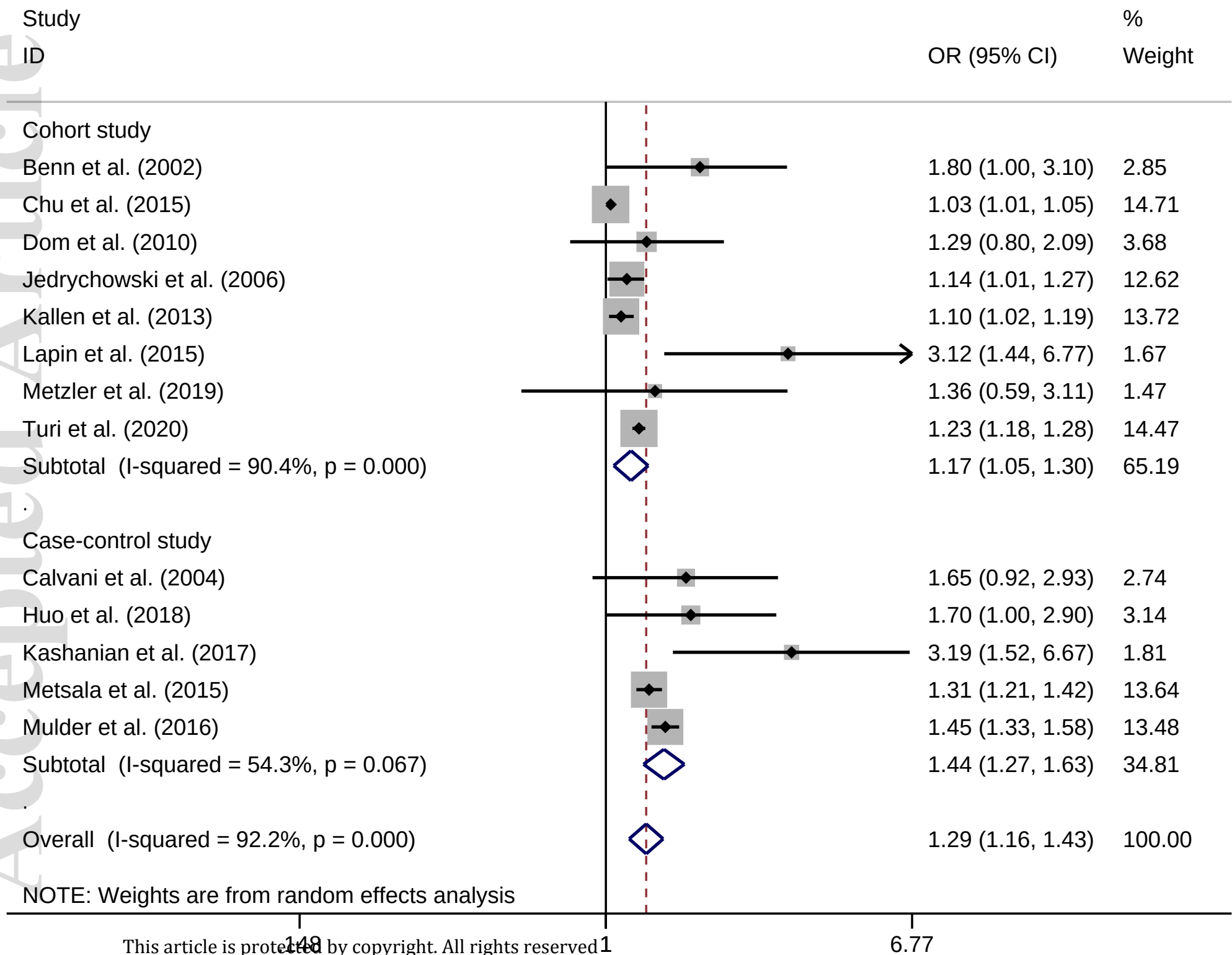
Metsälä et al. ³² (2013)	Finland	32474	Database for antibiotic prescriptions	0-2	Database for diagnosis of cow's milk allergy	Cow's milk allergy, OR: 1.21 (1.14-1.28)	Maternal age, socioeconomic status, smoking during pregnancy, previous deliveries, multiple pregnancies, and child's use of antibiotics.
Metsälä et al. ³³ (2015)	Finland	13380	Database	≥ 3	Database	Asthma, OR: 1.31 (1.21-1.42)	Maternal asthma, previous deliveries and gestational age.
Mulder et al. ³⁵ (2016)	Netherlands	26278	Database for antibiotic prescriptions	0-5	Database for asthmatic prescriptions	Asthma OR: 1.45 (1.33-1.58)	Gender, maternal age at delivery, maternal use of acid-suppressive drugs, antidepressant drugs and drugs for atopic diseases during pregnancy.

Abbreviations: HR, hazard ratio; OR, odds ratio; RR, relative risk; BMI, body mass index

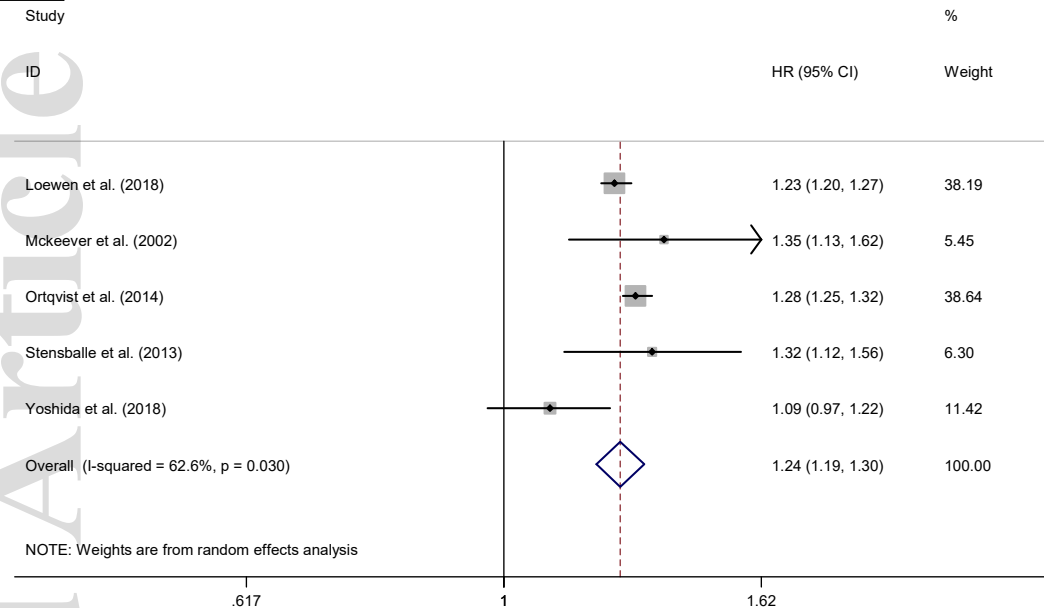


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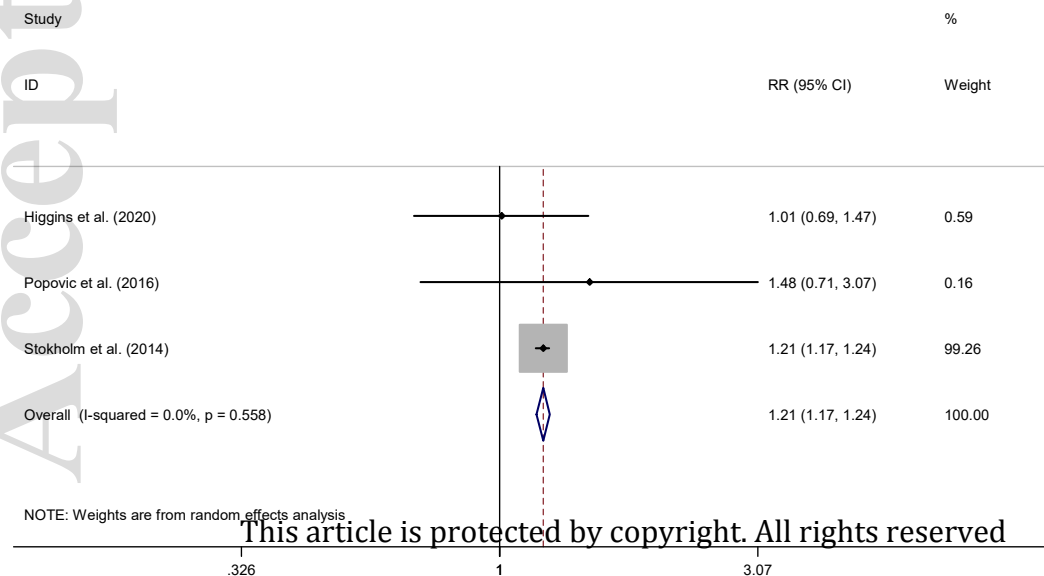




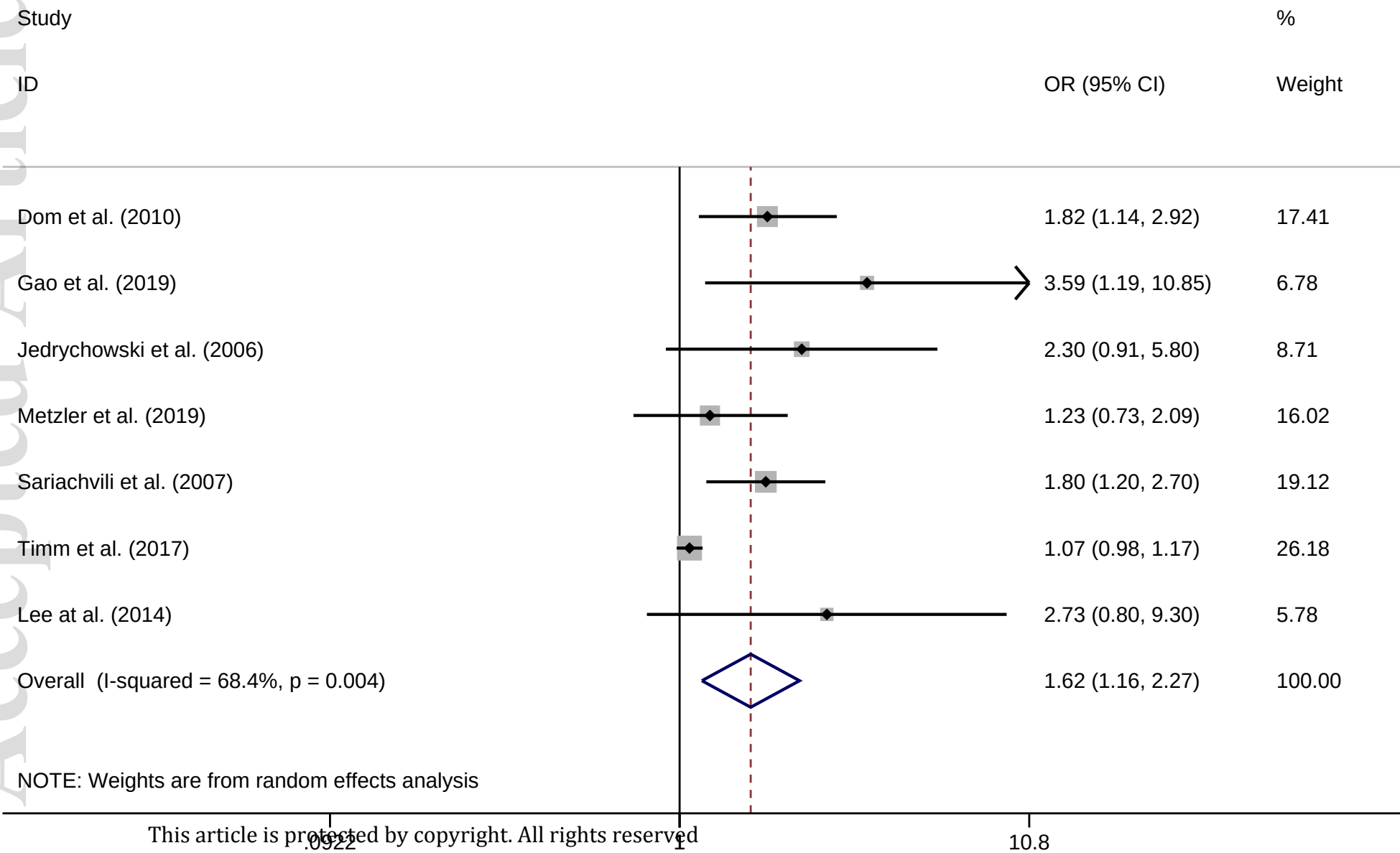
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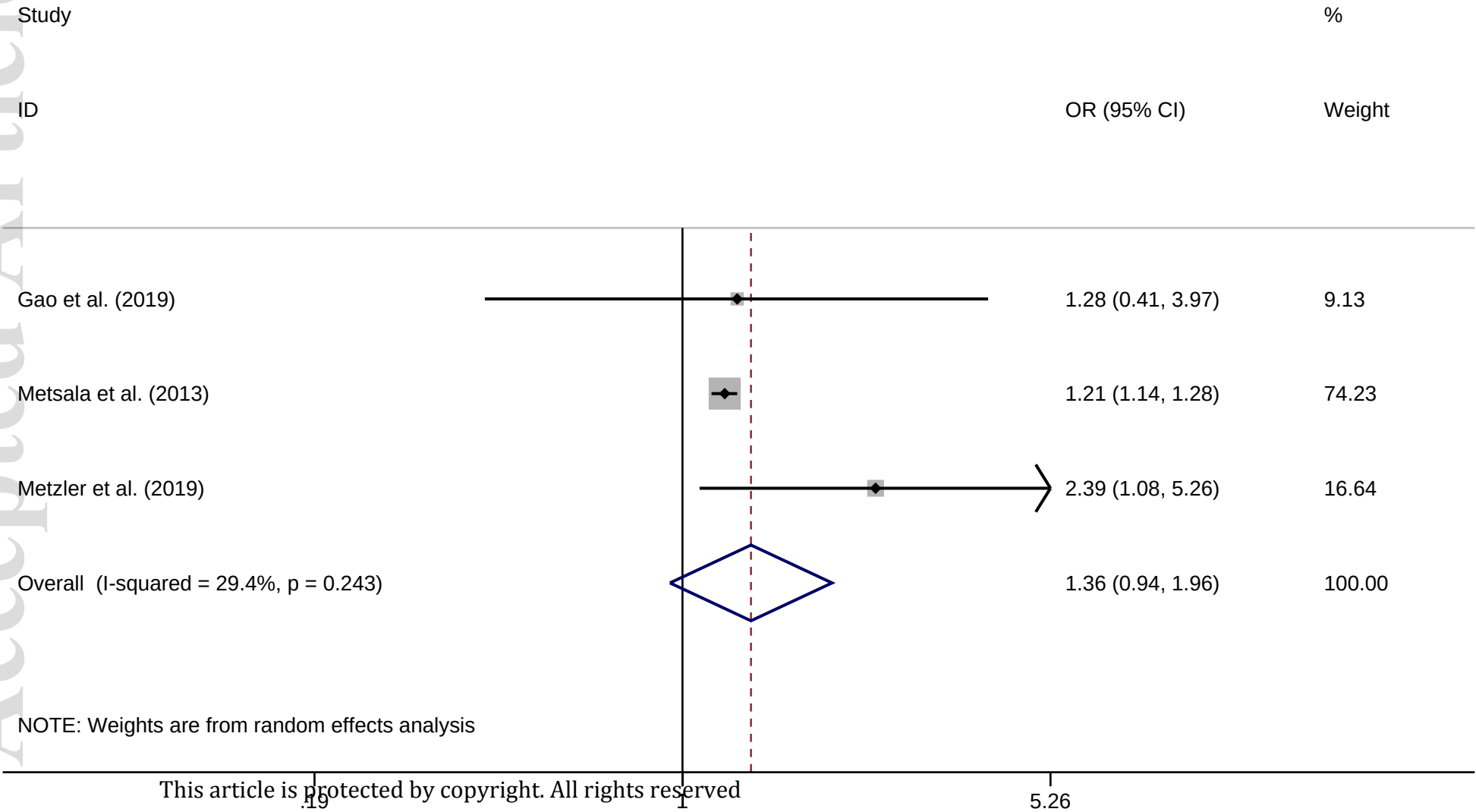


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Accepted Article





**Maternal antibiotic exposure during pregnancy and the risk of allergic diseases
in childhood: A meta-analysis**

SUPPORTING INFORMATION

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Supplementary Table 1. Search strategies for each database

Database	Search strategies	results
PubMed	Search: (((((((pregnancy[MeSH Terms]) OR (gestation)) OR (prenatal)) OR (maternal)) OR (in utero)) OR (antenatal)) AND (((((((anti-bacterial agents[MeSH Terms]) OR (antibiotic)) OR (antibacterial)) OR (antimicrobial)) OR (bacteriocidal agents)) OR (bacteriocides)) OR (antimycobacterial agents))) AND (((((((((((((((asthma[MeSH Terms]) OR (eczema[MeSH Terms])) OR (dermatitis, atopic[MeSH Terms])) OR (food hypersensitivity[MeSH Terms])) OR (allergy)) OR (atop*)) OR (bronchial asthma)) OR (allergic asthma)) OR (wheeze)) OR (wheezing)) OR (respiratory hypersensitivity)) OR (eczematous dermatit*)) OR (atopic dermatit*)) OR (atopic neurodermatit*)) OR (disseminated neurodermatit*)) OR (atopic eczem*)) OR (rash)) OR (Itchy rash)) OR (itching skin disease)) OR (food allergy))) AND (((((((child[MeSH Terms]) OR (children)) OR (childhood)) OR (neonatal)) OR (Infant)) OR (early life)))	1155
EMBASE	#1 'pregnancy'/exp OR gestation OR prenatal OR maternal OR 'in utero' OR antenatal #2 'antibiotic'/exp OR antibacterial OR antimicrobial OR 'bacteriocidalagents' OR bacteriocides OR 'antimycobacterial agents' OR 'anti-bacterial agents' #3 'asthma'/exp OR 'eczema'/exp OR 'atopic dermatitis'/exp OR 'food allergy'/exp OR allergy OR atop* OR 'bronchial asthma' OR 'allergic asthma' OR 'respiratory hypersensitivity' OR wheeze OR 'eczematous dermatit*' OR 'atopic dermatit*' OR 'atopic neurodermatit*' OR 'disseminated neurodermatit*' OR 'atopic eczem*' OR rash OR 'itchy rash' OR 'itching skin disease' OR 'food hypersensitivity' #4 'child'/exp OR children OR 'early life' OR childhood OR neonatal OR infant #5 #1 AND #2 AND #3 AND #4	1267
CENTRAL	#1 MeSH descriptor: [Pregnancy] explode all trees #2 gestation OR prenatal OR maternal OR in utero OR antenatal #3 #1 OR #2 #4 MeSH descriptor: [Anti-Bacterial Agents] explode all trees #5 antibiotic OR antibacterial OR antimicrobial OR bacteriocidal agents OR bacteriocides OR antimycobacterial agents #6 #4 OR #5 #7 MeSH descriptor: [Asthma] explode all trees #8 MeSH descriptor: [Eczema] explode all trees #9 MeSH descriptor: [Dermatitis, Atopic] explode all trees #10 MeSH descriptor: [Dermatitis, Atopic] explode all trees #11 allergy OR atop* OR bronchial asthma OR allergic asthma OR respiratory hypersensitivity OR wheeze OR eczematous dermatit* OR atopic dermatit* OR atopic neurodermatit* OR disseminated neurodermatit* OR atopic eczem* OR rash OR Itchy rash OR Itching skin disease OR food allergy #12 #7 OR #8 OR #9 OR #10 OR #11 #13 MeSH descriptor: [Child] explode all trees #14 children OR early life OR childhood OR neonatal OR Infant #15 #13 OR #14 #16 #3 AND #6 AND #12 AND #15	32

Supplementary Table 2. Assessment of quality of the studies according to the Newcastle-Ottawa Scale (NOS)

Author (year)	Study Design	Selection	Comparability	Exposure/Outcome	Score
Benn et al. (2002)	Cohort	**	**	***	7
Chu et al. (2015)	Cohort	**	**	***	7
Dom et al. (2010)	Cohort	***	**	*	6
Gao et al. (2019)	Cohort	****	**	**	8
Higgins et al. (2020)	Cohort	****	**	**	8
Jedrychowski et al. (2006)	Cohort	***	**	**	7
Källén et al. (2013)	Cohort	***	*	**	6
Lapin et al. (2015)	Cohort	***	**	**	7
Lee et al. (2014)	Cohort	****	*	***	8
Loewen et al. (2018)	Cohort	****	**	***	9
McKeever et al. (2002)	Cohort	****	**	**	8
Metzler et al. (2019)	Cohort	***	**	**	7
Örtqvist et al. (2014)	Cohort	***	**	**	7
Popovic et al. (2016)	Cohort	***	**	**	7
Sariachvili et al. (2007)	Cohort	****	**	***	9
Stensballe et al. (2013)	Cohort	****	**	**	8
Stokholm et al. (2014)	Cohort	***	**	**	7
Timm et al. (2017)	Cohort	***	*	**	6
Turi et al. (2020)	Cohort	***	**	***	8
Yoshida et al. (2018)	Cohort	****	*	***	8
Calvani et al. (2004)	Case-control	***	**	**	7
Huo et al. (2018)	Case-control	****	**	*	7
Kashanian et al. (2017)	Case-control	***	**	***	8
Metsälä et al. (2013)	Case-control	****	*	***	8
Metsälä et al. (2015)	Case-control	****	**	***	9
Mulder et al. (2016)	Case-control	****	*	***	8

Supplementary Table 3. Subgroup analyses of the association between maternal antibiotic use during pregnancy and the risk of childhood asthma/wheeze

Subgroup	No. of studies	OR (95% CI)		Heterogeneity	
		OR	95% CI	I ²	P
Overall	13	1.29	1.16-1.43	92.2%	<0.001
Study design					
Cohort study	8	1.17	1.05-1.30	90.4%	<0.001
Case-control study	5	1.44	1.27-1.63	54.3%	0.067
Study region					
Europe	8	1.28	1.14-1.43	75.3%	<0.001
USA	3	1.18	0.99-1.41	97.0%	<0.001
Asia	2	2.21	1.20-4.05	45.4%	0.176
Exposure assessment					
Parent-report or interview	8	1.27	1.08-1.50	67.4%	0.003
Database or records	5	1.29	1.16-1.43	86.7%	<0.001
Sample size					
<1000	5	1.70	1.13-2.54	92.2%	0.006
≥1000	8	1.25	1.11-1.41	94.8%	<0.001
Year of publication					
<2015	5	1.14	1.05-1.25	20.3%	0.285
≥2015	8	1.34	1.16-1.55	95.2%	<0.001
Child's age					
<3	2	1.75	0.66-4.64	84.3%	0.012
≥3	11	1.29	1.15-1.45	93.1	<0.001
Mother's age					
<29	5	1.29	1.08-1.53	94.9%	<0.001
≥29	4	1.36	1.12-1.67	75.8%	0.006
Types of antibiotics					
Penicillins	3	1.23	0.98-1.55	95.8%	<0.001
Sulfonamides and trimethoprim	3	1.23	0.87-1.71	82.9%	0.003
Tetracyclines	2	1.09	0.90-1.33	36.3%	0.210
Nitrofurantoin	2	1.05	0.95-1.17	0	0.572
Macrolides	2	1.30	1.11-1.52	0	0.712
Trimesters of pregnancy					
First trimester	4	1.13	1.05-1.21	64.5%	0.038
Second trimester	4	1.12	1.02-1.22	80.7%	0.001
Third trimester	4	1.17	1.01-1.35	91.9%	<0.001
Adjusted covariates (yes/no)					
Maternal age (yes)	8	1.27	1.11-1.44	94.3%	<0.001
Maternal age (no)	5	1.31	1.11-1.54	59.9%	0.041
Maternal allergy (yes)	11	1.29	1.14-1.46	90.9%	0.001
Maternal allergy (no)	2	1.26	0.96-1.65	95.4%	<0.001
Maternal smoking (yes)	7	1.18	1.04-1.33	91.5%	<0.001

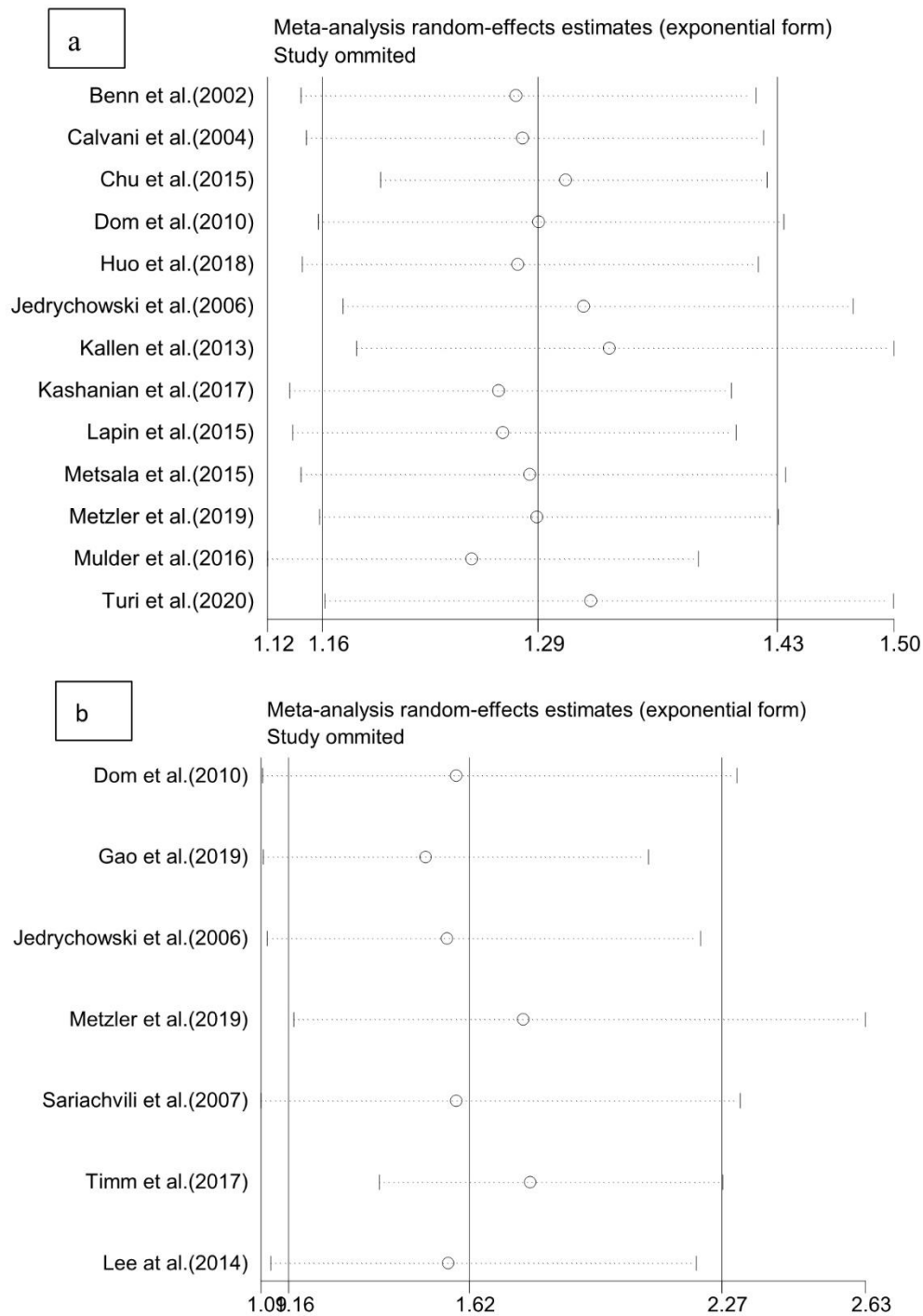
Maternal smoking (no)	6	1.38	1.20-1.57	72.6%	0.003
Delivery mode (yes)	3	1.29	1.16-1.43	0	0.481
Delivery mode (no)	10	1.30	1.14-1.49	91.8%	<0.001
Maternal education (yes)	6	1.22	1.18-1.27	0	0.429
Maternal education (no)	7	1.33	1.13-1.56	94.3%	<0.001

Abbreviations: OR, odds ratio

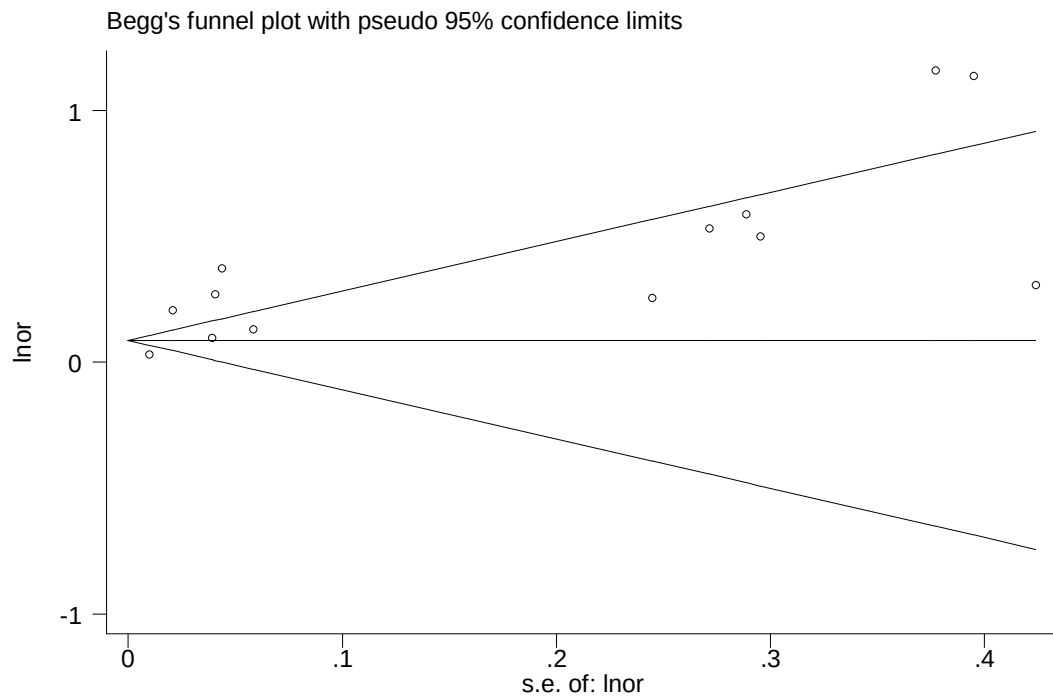
Supplementary Table 4. Subgroup analyses of the association between maternal antibiotic use during pregnancy and the risk of childhood eczema/atopic dermatitis

Subgroup	No. of studies	OR (95% CI)		Heterogeneity	
		OR	95% CI	<i>I</i> ²	<i>P</i>
Overall	7	1.62	1.16-2.27	68.4%	0.004
Study region					
Europe	5	1.46	1.06-2.01	68.8%	0.012
Asia	2	3.18	1.40-7.22	0	0.745
Exposure assessment					
Parent-report or review	6	1.57	1.12-2.20	70.6%	0.004
Database or records	1	2.73	0.80-9.30	NA	NA
Sample size					
<1000	5	1.97	1.49-2.59	0	0.770
≥1000	2	1.07	0.98-1.17	0	0.609
Year of publication					
<2015	3	1.85	1.38-2.48	0	0.889
≥2015	4	1.42	0.91-2.22	57.1%	0.072
Child's age					
<3	5	1.62	1.16-2.27	73.1%	0.005
≥3	2	1.52	1.04-2.23	15.6%	0.276
Mother's age					
<29	2	1.87	1.29-2.72	0	0.635
≥29	3	1.88	0.78-4.55	70.5%	0.034
Adjusted covariates (yes/no)					
Maternal age (yes)	4	1.94	1.45-2.58	0	0.639
Maternal age (no)	3	1.16	0.89-1.52	29.5%	0.242
Maternal allergy (yes)	5	1.75	1.36-2.24	0	0.444
Maternal allergy (no)	2	1.39	0.61-3.16	55.1%	0.135
Maternal smoking (yes)	6	1.57	1.11-2.21	70.2%	0.005
Maternal smoking (no)	1	2.30	0.91-5.80	NA	NA
Delivery mode (yes)	2	1.87	0.67-5.22	66.0%	0.086
Delivery mode (no)	5	1.62	1.09-2.39	72.8%	0.005
Maternal education (yes)	6	1.78	1.39-2.27	0	0.518
Maternal education (no)	1	1.07	0.98-1.17	NA	NA

Abbreviations: OR, odds ratio; NA, not available



Supplementary Figure 1. Sensitivity analysis of the included studies on the association between antibiotic exposure during pregnancy and allergic diseases in children (a: childhood asthma/wheeze; b: childhood eczema/atopic dermatitis).



Supplementary Figure 2. Begg's funnel plot of the included studies on the association between antibiotic exposure during pregnancy and childhood asthma/wheeze

Analyses of antibiotic exposure during pregnancy and risk of asthma/wheeze

Sensitivity analysis: The sensitivity analysis was conducted by removing one single study from the included studies on asthma/wheeze at each time and we did not find any study that might significantly affect the robustness of our findings (Supplementary Figure 1a).

Publication bias evaluation: We found that asymmetry appeared in Begg's funnel plot (Supplementary Figure 2). Similarly, the result of Egger's test ($P = 0.016$) also indicated the presence of significant publication bias.

Analyses of antibiotic exposure and risk of eczema/atopic dermatitis

Sensitivity analysis: The results indicated that no individual study significantly changed the summary OR for prenatal antibiotic use on childhood eczema or atopic dermatitis risk, demonstrating the results were robust (Supplementary Figure 1b). However, we found that after removing the study by Timm et al.¹, the heterogeneity index I^2 dropped sharply from 68.4% to 0, suggesting that it was an important source of heterogeneity.

Publication bias evaluation: There were only seven studies on eczema/atopic dermatitis included in the analysis, thus we did not evaluate publication bias since it was advised to analyze at least ten studies².

Reference

1. Timm S, Schlünssen V, Olsen J, Ramlau-Hansen CH. Prenatal antibiotics and atopic dermatitis among 18-month-old children in the Danish National Birth Cohort. *Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology*. 2017;47(7):929-936.
2. Higgins J. Cochrane handbook for systematic reviews of interventions. Version 5.1. 0 [updated March 2011]. The Cochrane Collaboration. 2011.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3-4
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5-6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6-7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7-8
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7-8
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8-10
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	8-10
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8-10
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	8-10
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10-13
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	13-14
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	15
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	15

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097.
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