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ORIGINAL PAPER

Early Life Antibiotic Exposure and the Subsequent Risk of Autism Spectrum Disorder and Attention Deficit Hyperactivity Disorder: A Systematic Review and Meta-Analysis

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Abstract
This study was conducted to assess this association between early life antibiotic exposure and the risk of autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) in later life. The results showed that early life antibiotic exposure was associated with an increased risk of ASD (OR = 1.13, 95% confidence interval (CI): 1.07–1.21) or ADHD (OR = 1.18, 95% CI: 1.1–1.27). However, this association for ASD (OR = 1.04, 95% CI: 0.97–1.11) or ADHD (OR = 0.98, 95% CI: 0.94–1.02) disappeared when data from sibling-matched studies were pooled. The statistically significant association between early life antibiotic exposure and ASD or ADHD in later life can be partially explained by unmeasured genetic and familial confounding factors.

Keywords Infection · Neurodevelopment · Risk · Systematic

Introduction

Human microbial colonization begins prior to birth, in utero, and continues to develop early in life, which is a critical period for gut microbial colonization (Lozupone et al., 2012). The recent proposal of a gut microbiota-brain axis has generated significant interest regarding the effects of bacterial microbiota composition on brain function (Cong, 2016). Recent research has supported the idea that interruption of bacterial intestinal colonization might promote permanent shifts in the microbial composition and result in abnormal neurodevelopment, thereby increasing the risk of neurodevelopmental disorders(Vuong, 2020). Mounting evidence from animal studies have supported the idea that early life antibiotic exposure causes long-term changes in gut microbiota composition and affects brain signaling pathways involved in social and emotional behavior(Hoban, 2016; Lach, 2020). Altered gut microbiota composition has been characterized in several neurodevelopmental disorders (Jiang, 2018b; Liu et al., 2019; Strati, 2016). Therefore, antibiotic use in early life may, hypothetically, contribute to the etiology of neurodevelopmental disorders.

Autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) are the two most common neurodevelopmental disorders, and their incidence has sharply increased in recent years (Lord et al., 2018; Posner et al., 2020). This increase has not been explained by any change in established risk factors; therefore, determining other potential risk factors for ASD and ADHD is important. In several epidemiological studies(Aversa, 2020; Axelsson, 2019a, 2019b; Bittker & Bell, 2018; Grossi et al., 2016; Hamad et al., 2018, 2019; Köhler-Forsberg, 2019; Lavebratt, 2019; Slob, 2020; Vargason et al., 2019; Wimberley, 2018), the long-term effects of antibiotic exposure on neurodevelopment were investigated; however, the reported results were inconsistent. In the earliest case–control study, Grossi et al. (Grossi et al., 2016) reported that antibiotic use during the first 3 months of life was associated with an increased risk of ASD; however, in three other studies (Bittker & Bell, 2018; Hamad et al., 2018; Lavebratt et al., 2019), ASD was

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reportedly not associated with antibiotic exposure early in life. In several studies (Aversa et al., 2020; Lavebratt et al., 2019; Slob et al., 2020), early life antibiotic exposure was also shown to increase the risk of ADHD later in life; however, the results were inconsistent based on further subgroup analysis. Due to the increasing prevalence of ASD and ADHD, determining the long-term effects of early life antibiotic use on neurodevelopment, including a possible effect on the incidence of either of these disorders, is important. Therefore, we conducted a systematic literature review and meta-analysis to assess the association between early life antibiotic exposure and the subsequent risk of neurodevelopmental disorders.

Methods

Data Sources and Search Strategy

Our systematic review was conducted by following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (Table S1) and the Meta-Analysis for Observational Studies in Epidemiology (MOOSE) checklist (Stroup, 2000). PubMed and EMBASE databases were searched to identify relevant studies written in English and published before November 2020. We used search terms relating to ASD, ADHD, antibiotic exposure, and study design and limited all results to studies on humans; Table S2 shows the full search strategy. In addition, the reference lists of relevant papers were manually searched for additional studies. Study review selection was independently completed by two reviewers, with any differences resolved by consensus and further discussion with a third reviewer. Because this was secondary data analysis, ethical approval was not required.

Selection Criteria

Observational studies were included if they met the following inclusion criteria: (1) peer-reviewed study with a case–control or cohort design; (2) the studies compared early life antibiotic exposure and non-exposure; (3) the included participants were 18 years or younger; (4) they explored whether early life antibiotic exposure increased the risk of subsequent risk of ASD or ADHD; and (5) they had sufficient available data to allow calculation of risk estimates if adjusted data were not provided. Case reports, case series, animal studies, editorials and reviews were excluded.

Data Extraction

Two authors independently extracted the data and any discrepancies were resolved by a third author. The extracted data included first author, publication year, study design, country, exposure age, ascertainment of antibiotic exposure, outcome measurement, sample size, adjusted confounders and study quality. The most-adjusted effect size estimate was used when more than one estimate was provided.

Risk of Bias and Quality Assessment

As recommended by the Cochrane Collaboration, the Newcastle–Ottawa Scale (NOS) was used to assess the risk of bias and study quality (Higgins, 2014). In brief, the scale has eight criteria, and the scores range from 0 (high risk of bias) to 9 (low risk of bias). A total star rating of ≤ 5 was considered a high risk of bias, a star rating between 6 and 7 intermediate risk of bias, and a maximal star rating ≥ 8 was considered low risk of bias.

Outcome Assessment

Our primary analysis calculated the odds ratios (ORs) of ASD or ADHD in children with early life exposure to antibiotics and compared these results with comparable data from children with no exposure. A secondary analysis was performed to rule out genetic and familial confounding. We compared outcomes between siblings or twins exposed or unexposed to antibiotics.

Statistical Analysis

All statistical analyses were conducted using Stata SE software (ver. 13.0; StataCorp, College Station, TX, USA). A random-effects model was used to pool the odds ratios (ORs) and 95% confidence intervals (CI) of individual studies; such models are optimal in terms of allowing the results to be generalized because they can account for potential heterogeneity (Higgins et al., 2003). Random-effects models were used to analyze pooled effects when significant heterogeneity was present; otherwise, fixed-effects models were used. An I^2 of $> 50\%$ or a P-value of < 0.05 for the Q-statistic was considered to indicate significant heterogeneity. Splitting one study into several estimates confers substantially more weight to that study in the meta-analysis, especially in a random-effects model. Therefore, we used a fixed-effects model to produce a pooled OR if ≥ 2 estimates from one study were provided; this pooled OR was then included in the meta-analysis. The presence of publication bias was quantitatively assessed using Egger's regression test and qualitatively assessed by visual inspection of funnel plots of the logarithm of OR versus the standard error (Egger et al., 1997). Statistical significance was defined using a two-sided test and P-value < 0.05.

Results

Search Results

Figure 1 shows the number of articles excluded at each stage of eligibility assessment with the reasons for exclusion. After using the keywords, 1812 potentially eligible articles were initially identified in searches of the two databases. Among these, 1559 articles, as well as 253 duplicates, were excluded after reading the titles and abstracts; 35 citations were eligible for full-text screening. After close review, 10 publications were excluded due to lack of usable data and failure to meet the inclusion criteria. From these studies, three studies included children born in Denmark. We included these three studies in the meta-analysis because they had been conducted by different investigators and used different patient eligibility criteria, although the study period of Axelsson et al. (Axelsson et al., 2019b)fully covered the periods of studies conducted by Wimberley et al.(Wimberley et al., 2018) and Köhler-Forsberg et al.(Köhler-Forsberg et al., 2019). Finally, 12 studies (10 on ASD and six on ADHD) were included in our analysis.

Study Characteristics

Table 1 shows the characteristics of the 12 studies in which the association between early life antibiotic exposure and ASD or ADHD risk was investigated; there were 10 cohort studies and two case–control studies involving more than 3,365,000 participants. The majority of the included studies were published in the past 2 years, with sample sizes ranging from 113 to 990,098. All studies were performed among Western populations. The exposure to antibiotics during childhood was assessed using parental interviews or a drug prescription database. The extent of adjustment for potential clinical risk factors varied considerably across studies. Based on the methodological quality assessment scores, eight studies were of high quality and the mean score of the 12 studies was 8.2. The breakdown of scores is shown in Table S3 and S4.

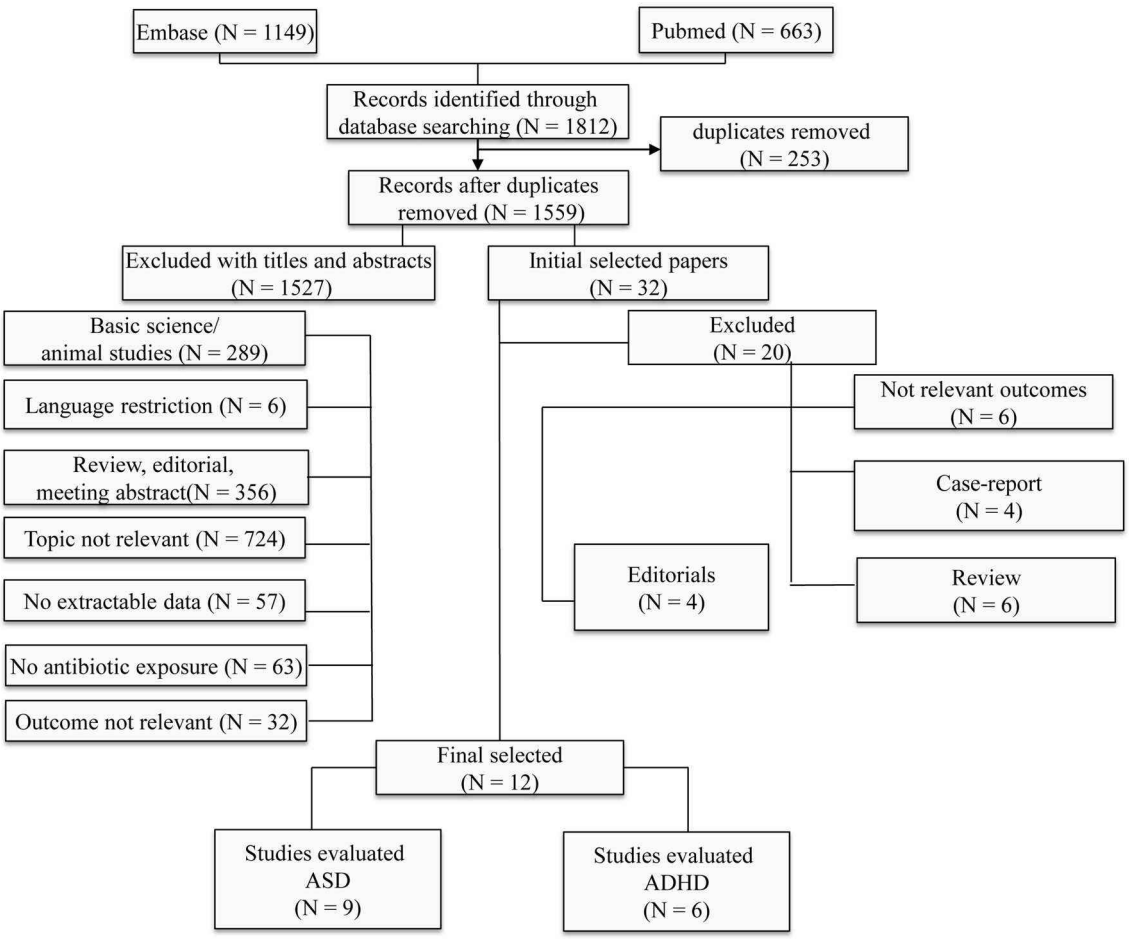

Fig. 1 Flow chart of the studies considered and finally selected for review

Table 1 Characteristics of the included studies

Author, year	Location, setting	Study design/ Born period of the children	Age	Exposure age	Ascertainment of antibiotic exposure	Outcome reported	Outcome measurement	Source of diagnosis	Number of particiants	Confounding Adjusted (Yes/No)	Quality
Grossi et al, 2016	Italy, hospital-based	Case–control, NA	< 15	< 3 months	Parental interview	ASD	DSM-5	Clinical diagnoses	45 cases 68 controls	No	5
Bittker et al., 2018	USA, population-based	Case–control, NA	< 12	< 2 years	Parental interview	ASD	Parental interview	Clinical diagnoses	1,001 cases 514 controls	Gender, age of the child, ethnicity, Midwest, south, maternal education, age of the mother at the birth of the child, and relationship	6
Hamad et al, 2018	Canada, population-based	Cohort, 1998–2016	< 14	< 1 year	Drug Program Information Network	ASD	ICD-9 or ICD-10	Admin data	Overall Cohort: Exposed 93,415 Non-exposed 120,125 Sibling Cohort: Exposed 39,224 Non-exposed 40,480	Sex, region, health care access, SES, maternal age at delivery, maternal medical conditions, prenatal antidepressants use, size for gestational age, childhood medical conditions, birth complications, mode of delivery, multiple birth, breastfeeding initiation, year of birth, season of birth and birth order	9
Wimberley et al., 2018	Denmark, population-based	Cohort, 1997–2008	< 15	< 8.6 year	Danish National Prescription Registry	ASD	ICD-10	Admin data	Exposed 8,494 Non-exposed 404	Sex, age, maternal, and paternal age at birth, gestational age, parental psychiatric disorders prior to birth, somatic hospitalization during the previous year, allowing different baseline hazards for each calendat period and parity	9
Köhler-Forsberg et al., 2019	Denmark, population-based	Cohort, 1995–2012	< 23	< 23	Danish National Prescription Registry	ASD ADHD	ICD-10	Admin data	4,078 ASD cases; 14,168 ADHD cases	Age, sex, Charlson Comorbidity Index, highest parental educational level, any parental mental disorder since 1969, hospitalization for infection, and other previous mental disorders	9

Table 1 (continued)

Author, year	Location, setting	Study design/ Born period of the children	Age	Exposure age	Ascertainment of antibiotic exposure	Outcome reported	Outcome measurement	Source of diagnosis	Number of particiants	Confounding Adjusted (Yes/No)	Quality
Axelsson et al., 2019b	Denmark, population-based	Cohort, 1997–2010	< 15	< 2 years	Danish National Prescription Registry	ASD	ICD-10	Admin data	Exposed 188,183 Non-exposed 483,423 439,718 full-sibling families	Childhood antibiotics use, mode of delivery,maternal age at birth,parental age difference,parental education,maternal marital status,maternal smoking,infant sex,5-min Apgar score,instrument use at delivery,use of continuous positive airway pressure or ventilator,asphyxia,parental epilepsy,preeclampsia or hypertension,gestational diabetes, parity, maternal antibiotics use during the pregnancy, maternal infections during the pregnancy, parental psychiatric history	9
Axelsson et al, 2019a, b	Denmark, population-based	Cohort, 1997–2010	< 17	< 2 years	Danish National Prescription Registry	ADHD	ICD-10	Admin data	Exposed 188,184 Non-exposed 120,125 442,002 full-sibling families	Maternal age at birth, parental age difference, parental education, maternal marital status, maternal smoking, infant sex, 5-min Apgar score, instrument use at delivery, use of CPAP or ventilator, asphyxia, parental epilepsy, preeclampsia or hypertension, gestational diabetes, parity, induction of labor, induction of contractions, maternal antibiotics use during the pregnancy, maternal infections during the pregnancy, parental ADHD history	9

Table 1 (continued)

Author, year	Location, setting	Study design/ Born period of the children	Age	Exposure age	Ascertainment of antibiotic exposure	Outcome reported	Outcome measurement	Source of diagnosis	Number of particiants	Confounding Adjusted (Yes/No)	Quality
Hamad et al, 2019	Canada, population-based	Cohort, 1998–2017	< 18	< 1 year	Drug Program Information Network	ADHD	ICD-9 or ICD-10	Admin data	Overall Cohort: Exposed 84,424 Non-exposed 103,181 Sibling Cohort: Exposed 33,374 Non-exposed 34,297	Sex, breastfeeding initiation, mode of delivery, preterm delivery, size for gestational age, maternal age at delivery, birth complications, birth order, year of birth, childhood medical conditions, prenatal infections, and prenatal antidepressant exposure	9
Lavebratt et al, 2019	Finland, population-based	Cohort, 1996–2012	< 18	< 2 years	Finnish Register on Reimbursement Drugs	ASD ADHD	ICD-10	Admin data	Exposed 735,730 Non-exposed 254,368	Maternal age, parity, maternal smoking during pregnancy, mother unmarried, mother born elsewhere than Finland, cesarean section, mother's inpatient care due to mental health disorders, mother's purchase of psychotropic drugs during pregnancy, mother's diagnoses related to systemic inflammatory disorders, multiple birth, offspring sex, perinatal health problems	9
Vargason et al, 2019	USA, population-based	Cohort, 2000–2005	< 15	< 3 years	OptumLabs® Data Warehouse	ASD	ICD-9	Admin data	3,253 cases 278,370 controls	Gender, race, Gastrointestinal-related diagnosis	8
Slob et al, 2020	Netherlands and Sweden, population-based	Cohort, 1989–2016	7–12	< 2 years	Prescribed Drug Register	ASD ADHD	CPRS-R CBCL	Admin data	Sibling Cohort: 25,781 from Netherlands 7946 from Sweden	Gender, delivery mode, educational attainment, birthweight, asthma	9
Aversa et al, 2020	USA, population-based	Cohort, 2003–2011	< 14	< 2 years	Records-linkage system	ASD ADHD	ICD-9 or ICD-10	Admin data	Exposed 10,220 Non-exposed 4,352	Male sex, birth weight, ethnicity, cesarean section, age, education, smoking, and antibiotic use during pregnancy	9

Table 1 (continued)

ADHD attention deficit hyperactivity disorder, *ASD* autism spectrum disorder, *CPRS-R* Conners's Parental Rating Scale Revised, *CBCL* Child Behaviour Checklist, *DSM* The Diagnostic and Statistical Manual of Mental Disorders, *NA* not available, *ICD* International Classification of Diseases

Meta-Analysis

ASD

The association between exposure to antibiotics during childhood and ASD diagnosis was evaluated in 10 studies. As shown in Fig. 2A, early life antibiotic exposure was significantly associated with an increased risk of ASD development (risk OR = 1.13, 95% CI: 1.07–1.21; $I^2 = 60.8\%$). No evidence of publication bias was observed (Egger test, P = 0.37), as shown in Figure S1. In a sensitivity analysis that excluded two studies relying on interviews, the association remained unchanged (OR = 1.13, 95% CI: 1.06–1.2; $I^2 = 63.7\%$). Further analysis excluded the studies conducted by Wimberley et al. and Köhler-Forsberg et al.; the association remained unchanged (OR = 1.11, 95% CI: 1.04–1.18; $I^2 = 50.8\%$). Subgroup analyses found that both narrow- (OR = 1.04, 95% CI: 0.89–1.21; $I^2 = 82.9\%$) and broader- (OR = 0.99, 95% CI: 0.86–1.14; $I^2 = 80.2\%$) spectrum antibiotic exposure was not associated with an increased risk of ASD.

A sibling-matched analysis was conducted in three studies to control for confounding genetic and social factors. As shown in Fig. 2B, no significant difference was observed in the risk of ASD between siblings who were or were not exposed to antibiotics during childhood (OR = 1.04, 95% CI: 0.97–1.11; $I^2 = 0\%$). Subgroup analyses found both narrow- (OR = 1.07, 95% CI: 0.97– 1.18; $I^2 = 0\%$) and broader- (OR = 1.08, 95% CI: 0.99– 1.18; $I^2 = 80.2\%$) spectrum antibiotic exposures were not associated with an increased risk of ASD.

ADHD

The association between exposure to antibiotics during childhood and ADHD diagnosis was evaluated in six studies. As shown in Fig. 3A, early life antibiotic exposure was significantly associated with an increased risk of ADHD development (OR = 1.18, 95% CI: 1.1–1.27; $I^2 = 88.8\%$). Evidence of publication bias was not observed (Egger test, P = 0.80) as shown in Figure S2. In a subsequent analysis that excluded studies conducted by Köhler-Forsberg et al., the association remained unchanged (OR = 1.14, 95% CI: 1.07–1.22; $I^2 = 86.5\%$). Subgroup analyses found that both narrow- (OR = 1.11, 95% CI: 1.06, 1.16; $I^2 = 14.1\%$) and broader- (OR = 1.22, 95% CI: 1.18–1.27; $I^2 = 0\%$) spectrum antibiotic exposures were associated with an increased risk of ADHD.

A sibling-matched analysis was conducted in three studies to control for confounding genetic and social factors. As shown in Fig. 3B, no significant difference was observed in the risk of ADHD between siblings who were or were not exposed to antibiotics during childhood (OR = 0.98, 95% CI:

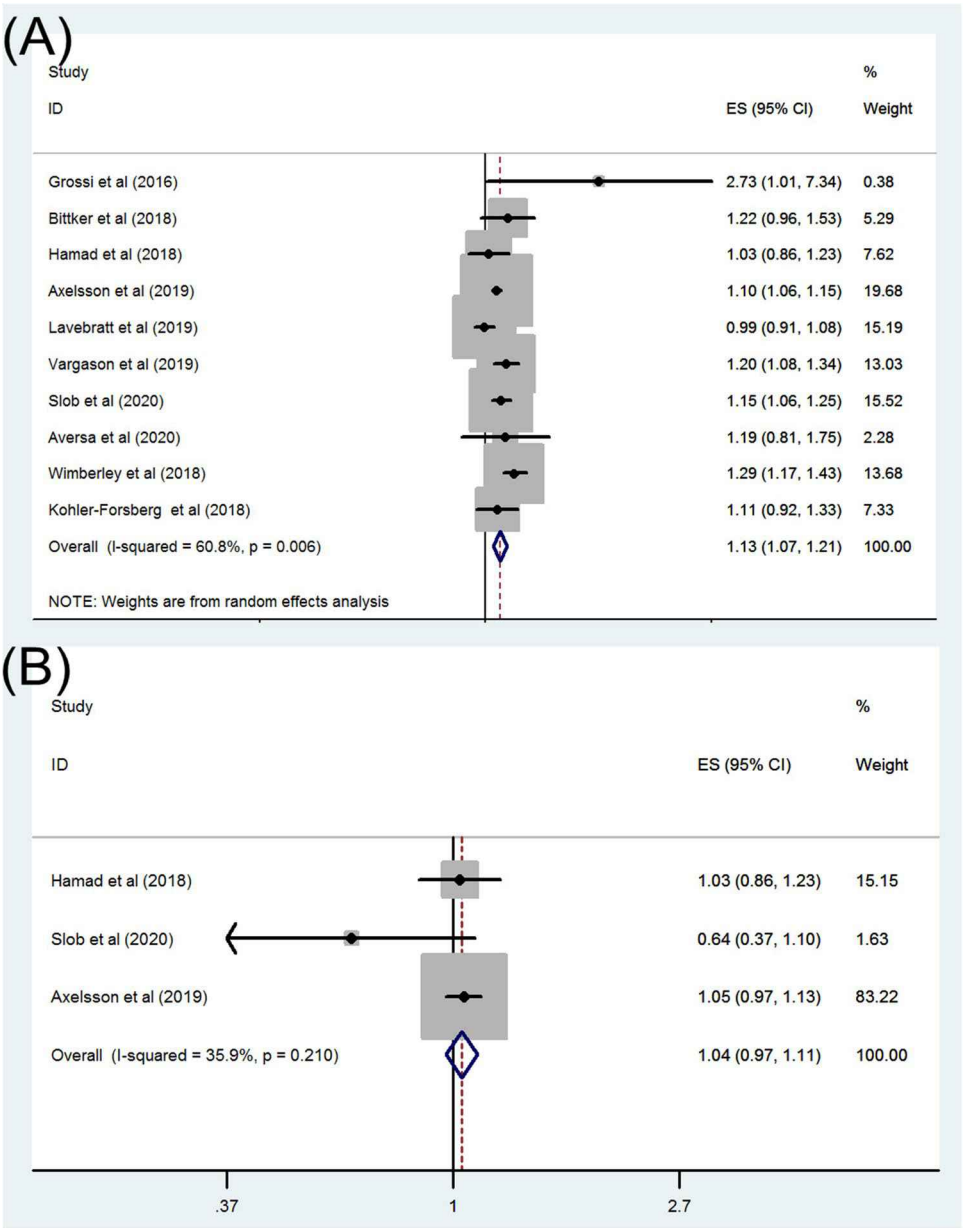

Fig. 2 Relative risk of subsequent ASD (**a**) unexposed vs exposed (**b**) unexposed vs exposed in sibling matched

0.94–1.02; $I^2 = 0\%$). Subgroup analyses found that both narrow- (OR = 1.01, 95% CI: 0.95–1.06; $I^2 = 0\%$) and broader- (OR = 1.06, 95% CI: 0.96–1.16; $I^2 = 42.8\%$) spectrum antibiotic exposures were not associated with an increased risk of ADHD.

Discussion

Our meta-analysis indicated that early life antibiotic exposure was associated with a subsequent increased risk of ASD or ADHD. However, such association was not found in the sibling-matched analysis, indicating that genetic and familial confounding factors may largely explain the observed association. However, because a small number of sibling-matched studies were included in our review, the results should be interpreted with caution.

The association between antibiotic use during childhood and neurodevelopmental disorders in later life has been a controversial topic in recent years (Champagne-Jorgensen et al., 2019). The observed behavior changes following exposure to antibiotics may be due to the effects of antibiotics on gut microbiota. Dysbiosis of gut microbiota could interfere with brain function through inflammation and the hypothalamic–pituitary–adrenal axis, thus affecting neurotransmission (Borre et al., 2014). In preclinical studies, the investigators reported that antibiotic-induced depletion of gut microbiota in early life results in long-lasting effects on

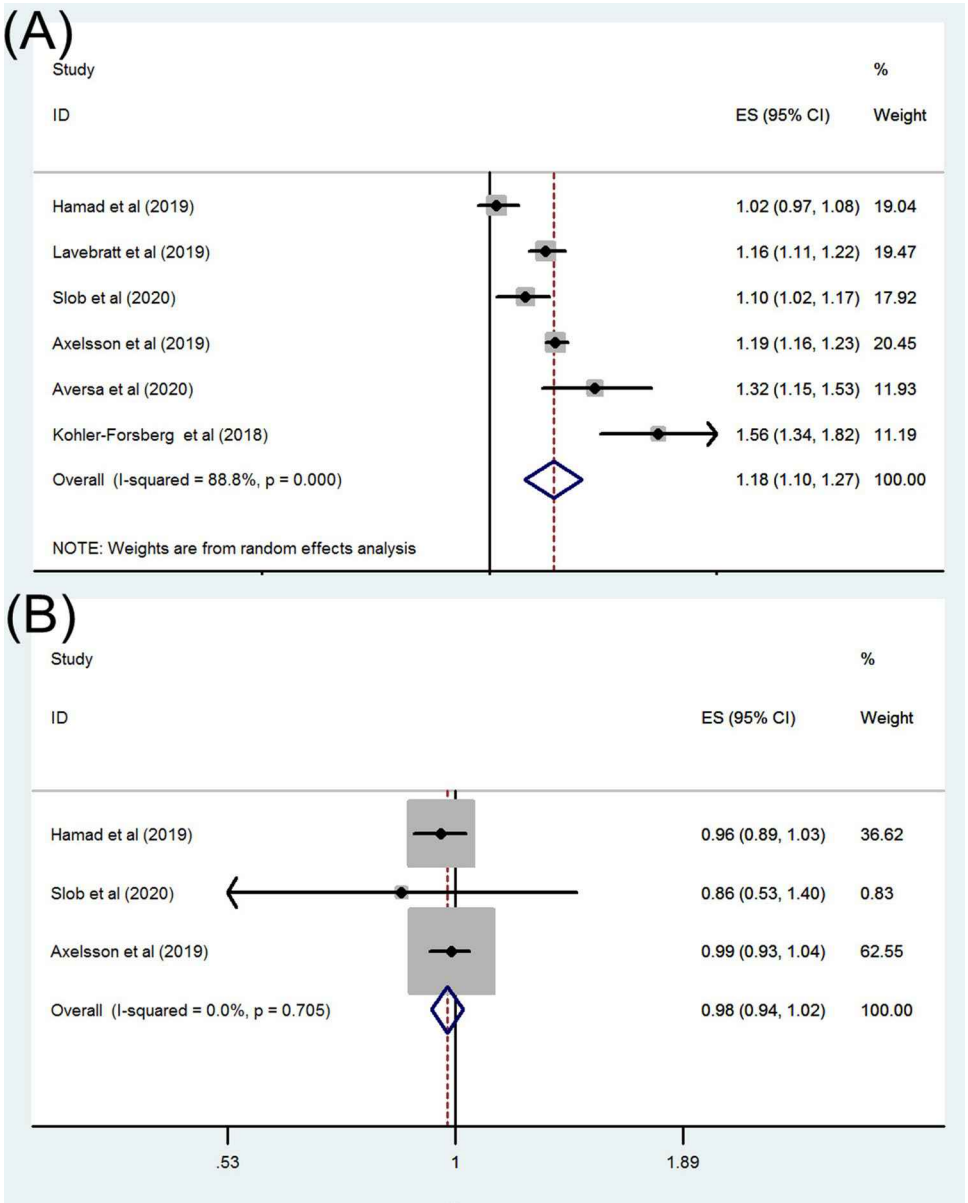

Fig. 3 Relative risk of subsequent ADHD (**a**) unexposed vs exposed (**b**) unexposed vs exposed in sibling matched

behavior, characterized by impaired attention, uncontrolled impulses and altered cognitive ability (Aguilera et al., 2013; Champagne-Jorgensen, 2020; Hoban et al., 2016). However, animal studies are not always generalizable to the clinical setting. In a previous systematic review (Lukasik et al., 2019), the association between antibiotic exposure during childhood and ASD was summarized but the inconsistencies regarding this association were not addressed.

ASD and ADHD both have been shown to have a high heritability, estimated to range from 60 to 80% in twin studies (Lord et al., 2018; Posner et al., 2020). In previous studies, other parental psychiatric diseases were also reportedly associated with the development of ASD and ADHD (Jiang et al., 2018a; Manzari et al., 2019; Morales et al., 2018); thus, investigation of the association between antibiotic use during childhood and ASD or ADHD should take parental psychiatric conditions into consideration. Furthermore, several prenatal factors, such as smoking, maternal infection and maternal and perinatal health problems have been determined to be associated with the risk of neurodevelopmental disorders (Kim, 2020). In a more recent meta-analysis (Lee et al., 2019), a significantly increased risk of ASD was observed in children exposed to antibiotics. This meta-analysis included only four studies with small sample sizes, and omitted important studies regarding the influence of unmeasured confounders on the risk of ASD. Their results were consistent with our overall findings, but were slightly more precise, most likely because their population was almost fourfold larger. However, the significant association between antibiotic exposure and ASD risk disappeared when

we pooled data from the sibling-matched studies, which partially controlled for confounding familial and genetic factors. This condition was observed in our analysis of ADHD risk, suggesting that unmeasured confounding may largely explain the observed association. Importantly, only three studies were included in the sibling-matched analysis.

Our study included an additional four studies, which attempted to control for a wide range of potentially confounding factors. Although the association between antibiotic exposure and ASD was identified in our primary and sensitivity analyses, any observed association could be the result of genetic factors and due to confounding maternal characteristics. Therefore, the ideal control for unmeasured confounding factors is using a sibling-matched analysis design, which should minimize the effects of these factors on the observed association. Contrary to the primary analysis, our secondary analysis based on a sibling-matched study design found the relationship between exposure to antibiotics and ASD or ADHD disappeared, and the heterogeneity was reduced to 0% among three sibling-matched studies. Furthermore, subgroup analysis based on type of antibiotic did not show increased risk of ASD or ADHD rates in children exposed to antibiotics in early life, indicating the magnitude of microbiota changes may be too small to affect neurodevelopment. However, the sample sizes in the sibling-matched studies were small, and further studies are needed to verify these results.

Our study has several notable strengths as well as important limitations. To the best of our knowledge, this is the first systematic review and meta-analysis in which the effects of antibiotic exposure during childhood on the risk of ADHD in later life were evaluated. The main strength of this meta-analysis is the careful consideration of potential confounding factors, especially in subgroup analysis based on sibling-matched study conducted to exclude the potential genetic and familial confounding factors. Second, the heterogeneity of subgroup analysis was mild ($I^2 = 0\%$), which increased the strength of the pooled results. Third, most of the included studies were cohort studies, which are less prone to bias in terms of assessing exposure during childhood.

Nevertheless, the study had several limitations. First, the number of included studies in which ADHD risk was evaluated was small, specifically when subgroup analyses based on sibling-matched study were used. Second, all reviewed studies included Western populations and not subjects from Asian or African countries, which may have affected the generalizability of our findings. Third, sufficient data on the dose of antibiotics used in the included studies could not be extracted; therefore, any exposure parameter possibly associated with ASD or ADHD risk in later life could not be defined.

In conclusion, the findings in this systematic review and meta-analysis suggested that the associations between early life antibiotic exposure and ASD or ADHD risk may be overestimated because previous studies failed to control for unmeasured familial or genetic confounding factors. Future research should adjust for potential confounders and consider genetically sensitive designs, such as sibling comparisons or twin and adoption studies.

Author Contribution H.Y.J. and H.Y.Y. conceived the study and revised the manuscript critically for important intellectual content. Y.Y.Z, L.Y.P. and X.Z. made substantial contributions to its design, acquisition, analysis and interpretation of data. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare no competing interest.

Ethical Approval No ethical approval was required for this review as all data were already published in peer-reviewed journals.

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