BMJ Open

Prenatal exposure to antibiotics and the risk of orofacial clefts: a protocol for a systematic review and meta-analysis of observational studies

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2024-092019
Article Type:	Protocol
Date Submitted by the Author:	05-Aug-2024
Complete List of Authors:	Nagata, Abir; Graduate School of Medicine, Osaka University Rahman, Md. Shafiur ; Hamamatsu University School of Medicine, Research Centre for Child Mental Development; United Graduate School of Child Development, Osaka University, Kanazawa University, Hamamatsu University School of Medicine, Chiba University, and University of Fukui, Suita, Japan Rahman, Mahfuzur; Graduate School of Public Health, St. Luke's International University Nakagawa, Takatoshi; Graduate School of Medicine, Osaka University Sharmin, Salma; University Dental College and Hospital, Dhaka, Bangladesh, Department of Dental Public health Ohnishi, Kazunari ; Graduate School of Public Health, St. Luke's International University Rahman, Mahbubur; Graduate School of Public Health, St. Luke's International University
Keywords:	Anti-Bacterial Agents, Fetal medicine < OBSTETRICS, EPIDEMIOLOGY, Child

TITLE

Prenatal exposure to antibiotics and the risk of orofacial clefts: a protocol for a systematic review and meta-analysis of observational studies

Abir Nagata[1], MD; Shafiur Rahman[2,3], MD; Mahfuzur Rahman[4]; Takatoshi Nakagawa[1]; Salma Sharmin[5]; Kazunari Onishi[4]; and Mahbubur Rahman[4]

[1]Graduate School of Medicine, Osaka University, Osaka, Japan

[2]Research Centre for Child Mental Development, Hamamatsu University School of Medicine, Hamamatsu, Japan

[3]United Graduate School of Child Development, Osaka University, Kanazawa University, Hamamatsu University School of Medicine, Chiba University, and University of Fukui, Suita, Japan

[4]Graduate School of Public Health, St. Luke's International University, Tokyo, Japan

[5]Department of Dental Public Health, University Dental College and Hospital, Dhaka, Bangladesh

E-Mails:

Abir Nagata: abir.med@osaka-u.ac.jp ; MD Shafiur Rahman: srahman@hama-med.ac.jp ; MD Mahfuzur Rahman: mdmahfuzur08@gmail.com ; Takatoshi Nakagawa: tnakagawa@r-derma.med.osaka-u.ac.jp ; Salma Sharmin: sharminbd_0027@yahoo.com ; Kazunari Onishi: kaznaly@slcn.ac.jp ; Mahbubur Rahman: rahman@slcn.ac.jp

CORRESPONDENCE

Abir Nagata, MPH, PhD

Graduate School of Medicine, Osaka University, Osaka, Japan

E-mail: abir.med@osaka-u.ac.jp

Tel: (81) 6-6879-3960

Word count

Abstract: 346 words

Full text: 2151 words

Tables: 1

Figures: 1

Supplemental material: 1

ABSTRACT

Introduction: Orofacial clefts (OFCs), including cleft lip (CL), cleft palate (CP), and combined cleft lip and palate (CLP), are among the most common craniofacial malformations in newborns, and present significant healthcare challenges. Emerging evidence has raised concerns regarding the potential impact of prenatal exposure to antibiotics on foetal development. Antibiotics prescribed during pregnancy—particularly to those that cross the placental barrier—may pose teratogenic risks. Previous studies investigating the association between prenatal antibiotic exposure and the risk of OFCs have yielded inconsistent results. However, no studies have yet attempted to summarise this evidence, highlighting the need for a comprehensive evaluation. This report describes a systematic review and meta-analysis protocol to retrospectively analyse the relationship between prenatal antibiotic exposure and the risk of developing OFCs, focusing on the role of antibiotic type and timing of exposure. The results of such a review will hopefully provide a comprehensive synthesis of the available evidence, helping to inform clinical practice and guide patient counselling regarding the use of antibiotics during pregnancy.

Methods and analysis: The planned systematic review and meta-analysis will adhere to the PRISMA-P guidelines, to ensure a comprehensive and systematic approach to summarising the available evidence on the topic. It will include observational studies (cohort, case-control, and cross-sectional) that investigate the association between prenatal antibiotic exposure and OFCs. The search strategy will cover major databases, including CINAHL, Cochrane Library, EMBASE, PubMed, Scopus, and Web of Science, using tailored search terms. A team of independent assessors will screen article titles, abstracts, and full texts. Any discrepancies will be resolved through discussions. Quality assessment will utilise the Newcastle-Ottawa Scale and GRADE criteria. Data extraction will focus on the study characteristics, participant details,

exposure specifics, and outcome measures. A random-effects meta-analysis will aggregate summary effect sizes, and heterogeneity will be assessed using I^2 and Q statistics.

Ethics and dissemination: Ethical approval is not required for this systematic review, as it relies on already published data. The findings will be disseminated through peer-reviewed journals and conference presentations, providing critical insights into clinical practice and public health policies regarding antibiotic use during pregnancy.

PROSPERO registration number: CRD42024565064

Key words: antibiotics; orofacial clefts; cleft lip; cleft palate

STRENGTHS AND LIMITATIONS OF THE STUDY

- This study will use a detailed search strategy across multiple databases (CINAHL, Cochrane Library, EMBASE, PubMed, Scopus, and Web of Science), as well as gray literature sources, while adhering to the PRISMA-P guidelines. The use of established quality assessment tools (the Newcastle-Ottawa Scale and GRADE criteria) will enhance its reliability and validity.
- Including only observational studies (cohort, case-control, and cross-sectional) provide real-world data regarding the association between prenatal antibiotic exposure and the presence of orofacial clefts (OFCs) in infants.
- Variability in the designs, populations, antibiotic types used, and timings of the included studies may lead to heterogeneity, complicating synthesis of the results. Unpublished or negative findings may be under-represented, potentially introducing publication bias.
- Limiting the search to English publications only may exclude relevant data, thereby affecting the comprehensiveness of the review. The review's conclusions will depend on the methodological quality and reporting standards of the included studies, which may vary.

INTRODUCTION

Orofacial clefts (OFCs) are among the most common craniofacial malformations in newborns and present significant healthcare challenges because of their complex aetiology and multifaceted impact on health [1–3]. They affect an estimated 4.6 million individuals globally, resulting in a burden of ~529,758.92 disability-adjusted life years (DALYs) [4]. OFCs—which include cleft lip (CL), cleft palate (CP), and combined cleft lip and palate (CLP)—result from disruptions in the normal development of the orofacial region during embryogenesis [2], and are specifically influenced by a combination of genetic, environmental, and maternal lifestyle factors [2,3,5]. Environmental influences such as maternal smoking, alcohol consumption, nutritional deficiencies, and medication use during pregnancy, have also been implicated in the pathogenesis of OFCs [5–7]

Recent research has raised concerns regarding the potential impact of prenatal exposure to antibiotics on foetal development [8–10]. Antibiotics are commonly prescribed during pregnancy to manage infections that, if left untreated, can pose significant risks to both the mother and the developing foetus [11–13]. However, the teratogenic potential of antibiotics, particularly those that cross the placental barrier, remains a topic of considerable debate and investigation [14]. Potential mechanisms through which antibiotics may contribute to the development of OFCs include the disruption of normal cellular processes, interference with folate metabolism, and induction of oxidative stress in the developing embryo [15].

Several studies have suggested an association between prenatal antibiotic exposure and an increased risk of OFCs [16–19], although current evidence on the matter is inconsistent overall [20–23], varying significantly across different antibiotic classes, dosages, and timings of exposure. The current body of literature on this topic is characterised by heterogeneity in terms

of study designs and populations, which may be due to differences in study populations, classifications of antibiotic exposure, and control of confounding variables. Therefore, a systematic approach to synthesising this evidence is essential for drawing robust and generalisable conclusions. Understanding the potential teratogenic effects of antibiotics is crucial for clinical practice and public health. Therefore, the following proposed study is not only important for informing clinical practice and guiding patient counselling, but also pivotal for shaping public health policies and future research trajectories in prenatal care and teratology.

OBJECTIVES

1. To systematically review observational studies investigating the relationship between prenatal antibiotic exposure and the risk of developing OFCs, with a particular focus on elucidating the role of antibiotic type, dosage, and timing of exposure.
2. To conduct a meta-analysis that quantitatively synthesises data from individual studies, providing a robust estimate of the association between prenatal antibiotic exposure and the risk of OFCs, while accounting for potential moderating factors.

METHODS

Protocol development

The protocol for this study aligns with the guidelines established by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P) [24,25] detailed in the PRISMA-P checklist (Supplement). The registration code for this review protocol (CRD42024565064) will be accessible on the PROSPERO International Prospective Register of Systematic Reviews. Any updates to the protocol and review process will be promptly reflected in the PROSPERO registration.

ELIGIBILITY

Inclusion and exclusion criteria

Participants

The participants in this study will be mother-child pairs. We will include all pregnant individuals regardless of age, ethnicity, or health status. We will include studies with explicit documentation of antibiotic exposure during pregnancy—including prescription records, patient self-reports, and medical notes confirming antibiotic use. The exclusion criteria include: studies lacking specific information regarding the pregnancy status of the participants, or those with ambiguous details regarding the timing and confirmation of pregnancy. We will also exclude studies in which antibiotic exposure is not explicitly linked to the pregnancy period, or where such exposure is inferred but not documented.

Exposure

Exposure to any class of antibiotics during pregnancy—including (but not limited to) penicillins, cephalosporins, macrolides, tetracyclines, fluoroquinolones, and sulfonamides—will be noted. We will include studies that provide details regarding the type of antibiotic (specific name or class), frequency of intake and trimester during which the exposure occurred. This detailed information is crucial for understanding the potential dose-response relationships and most critical periods of foetal development. However, studies with grouped antibiotic exposures that do not distinguish between different antibiotics or classes will be excluded. Reports with unclear or inconsistent information regarding the frequency, dosage, or timing of antibiotic exposure during pregnancy will also be excluded.

Comparator/Control

Groups of pregnant individuals who did not receive any antibiotics during pregnancy will serve as a baseline control group for comparisons. We will exclude studies that lack a well-defined control or comparison group, as well as those where the control group includes individuals with antibiotic exposures that are similar to those of the treatment groups (thus failing to provide a clear contrast for the analysis).

Outcomes

The primary focus of this study will be on OFCs, including both syndromic and non-syndromic CL, CP, and combined CLP. All OFC diagnoses should be based on clinical examinations, medical records, or standardised screening protocols—either prenatally, at birth, or within a defined postnatal period (up to one year). Studies that focused on outcomes unrelated to OFCs, or speculative studies, will be excluded.

Types of studies

Observational studies (cohort, case-control, and cross-sectional) that quantitatively assessed the relationship between prenatal antibiotic exposure and OFCs will be included. All included studies must have a sound methodological design, including well-defined populations, clear exposure and outcome measures, and appropriate statistical analyses. We will exclude case reports and case series, as they often lack generalisability, as well as reviews, editorials, commentaries, and animal studies because they typically do not provide original empirical data. Studies with methodological flaws such as inadequate sample sizes or lack of statistical rigor will also be excluded.

Search strategy and study selection

Our search methodology has been meticulously designed to identify both published and unpublished studies. This comprehensive approach covers electronic repositories, conference records, virtual platforms, scholarly dissertations, and (if necessary) direct correspondences with primary authors. The search will be limited to articles published in English. We will search databases including CINAHL, Cochrane Library, EMBASE, PubMed, Scopus, and Web of Science. Index terms and keywords will be carefully tailored to the unique characteristics of each database. Google Scholar will also be searched for gray literature and ongoing studies. The following search terms will be combined and adapted as needed to meet the database specifications: ("antibiotics" OR "antimicrobial agents" OR "anti-infective agents" OR "antibacterial drugs" OR "broad spectrum antibiotics" AND "prenatal exposure delayed effects" OR "maternal exposure" OR "intrauterine exposure" OR "gestational drug exposure") AND ("orofacial clefts" OR "cleft lip" OR "cleft palate" OR "congenital defects" OR "birth defects" OR "congenital anomalies" AND ("observational study" OR "longitudinal study" OR "retrospective study" OR "prospective study" OR "epidemiological research". Both MeSH terms and other appropriate subject headings will be used in the database search. The search strategy for the databases is illustrated in **Table 1**.

A team of three independent assessors will conduct the search. The retrieved studies will be imported into Rayyan (https://rayyan.ai/), a systematic review software platform [26]. Initially, two independent assessors will assess the suitability of the titles and abstracts of all screened publications, followed by full-text examinations in accordance with our inclusion criteria. Two assessors will concurrently evaluate the abstracts and full-text materials, and any discrepancies will be resolved through comprehensive discussions. **Figure 1** illustrates the screening process.

In cases where significant disparities persist, the ultimate resolution will be entrusted to the other assessors.

Quality assessment

Two assessors will independently evaluate the methodological rigor of the included studies using the Newcastle-Ottawa Scale (NOS) to assess the quality of the non-randomised studies included in the meta-analyses. Additionally, two assessors will independently apply the GRADE assessment to categorise the quality and strength of evidence in each publication as high, moderate, low, or very low, in terms of its primary outcomes. Any discrepancies between assessments will be resolved through constructive discussions. The assessment of methodological quality will be integrated into the discussion of the respective study findings.

Data extraction

Two assessors will independently review the included studies and extract relevant information using a pre-designed form. Discrepancies will be discussed until agreements are reached. The collected information will include details, such as author names, publication year, study location, study type (cross-sectional, case-control, or cohort), participant details (sample size, sampling methods, sex, and age), questionnaire type, and statistical outcomes (including effect measures, confidence intervals, and p-values). The two reviewers will use the STROBE checklist for observational studies to assess the quality of each study [27].

Data analysis and synthesis

The findings will be comprehensively presented using tables and figures. The effect size (ES) of interest will be relative risk (RR). In cases where the ES is not present, an unadjusted RR will be estimated from contingency tables. Odds ratios (ORs) will be converted to RRs using appropriate

formulae. Both fixed-effect and random-effects meta-analyses will be used to estimate the pooled ESs along with their associated 95% confidence intervals. Significance levels will be documented. Inter-study variability, measured using the Cochrane Q or I^2 statistics will be explored, as well as potential impacts from smaller studies. I^2 values of 25%, 50%, and 75% will be assumed to represent low, moderate, and high heterogeneity, respectively. The significance of heterogeneity will be determined via χ^2 values for Q statistics, with $p<0.05$. If the level of between-study heterogeneity is higher ($I^2 > 75\%$), a random-effects meta-regression will be performed to identify the potential moderators. A leave-one-sample-out validation will be used to explore the influences of each included study on the pooled ES. Funnel plots will be used to detect potential publication bias and small-study effects. $p<0.05$ will be considered indicative of statistically significant publication bias. For outcomes with more than 10 individual studies, we will use Egger's regression test to assess asymmetry. The combined effect size will be visually represented using a forest plot. If the number of studies included is insufficient (<3), a narrative review of the study findings will be presented instead of a meta-analysis.

Sensitivity analyses on primary outcomes will be conducted, and will involve excluding studies with a high risk of bias or incomplete data. Efforts will be made to contact researchers or study sponsors to obtain any missing information. If this is not possible, established methods for estimating missing data using multiple imputation will be applied. The validity of imputed data will be assessed through a sensitivity analysis. Subgroup analyses will explore the effects of timing of exposures, types and dosages of medication, and socio-economic status. Furthermore, we will define groups based on exposure to different classes of antibiotics and the timing of antibiotic administration during pregnancy, and conduct a comparative analysis across various exposure scenarios. All statistical analyses will be conducted in Stata version 18 (StataCorp).

DISCUSSION

This systematic review and meta-analysis protocol represents the first comprehensive effort to investigate the association between prenatal antibiotic exposure and the risk of OFCs. Its findings are expected to provide critical insights into medication safety during pregnancy and its potential teratogenic effects on foetal development, particularly regarding the occurrence of OFCs. Our conclusions will be based on the combined results of the included studies, presented through quantitative analysis or narrative synthesis.

Understanding the relationship between prenatal antibiotic exposure and OFCs is crucial for clinical decision-making and patient counselling. If a significant association is found, it will highlight the need for careful consideration of antibiotic prescriptions during pregnancy. This could lead to the development of guidelines and protocols aimed at minimising unnecessary antibiotic use and selecting safer alternatives when treatment is necessary. The results will also hopefully ensure that healthcare providers are better equipped to inform expectant mothers about the potential risks and benefits of antibiotic use during pregnancy, thereby facilitating more informed choices.

The major strengths of this planned systematic review lie in its rigorous methodological approach, adherence to the PRISMA-P guidelines, and use established quality assessment tools such as the Newcastle-Ottawa Scale and GRADE criteria. However, several methodological challenges must also be addressed. First, the included studies are likely to vary in their designs, populations, antibiotic types, dosages, and timing of exposure, which may introduce significant heterogeneity. This variability can complicate the synthesis of the results and limit the generalisability of our findings. Subgroup and sensitivity analyses will be crucial to addressing these issues. Second, the exclusion of non-English publications and the potential under-

representation of unpublished or negative findings may also introduce publication bias. Funnel plots and Egger's tests will be used to assess and mitigate this bias; however, its presence cannot be entirely ruled out. Third, the conclusions drawn from this review will depend on the methodological quality and reporting standards of the included studies. Variations regarding how antibiotic exposure and OFCs are defined and measured may impact the robustness of the findings. The use of the GRADE criteria will help evaluate the strength and quality of the evidence, providing a clearer understanding of the confidence that can be placed in the results. Nevertheless, this review is expected to identify gaps in the current literature and suggest areas for future research.

ETHICS AND DISSEMINATION

Obtaining ethical approval was not deemed necessary for this study, as it pertains to a protocol for a systematic review that relies on published and therefore publically available data. The findings of this study will be communicated through peer-reviewed articles and presentations at conferences.

ADDITIONAL INFORMATION

Author contributions: AN, MSF, and MMR conceived of the study's concept and drafted the initial version. TK, SS, KO, and MR provided guidance to the research teams. All of the authors participated in drafting and revising the manuscript, formulating the review questions, and designing the study. The final version of this manuscript has been read and approved by all of the authors.

Conflict of Interest Disclosures: The authors declare no competing interests.

Funding/Support: This research did not receive any specific funding from public, commercial, or nonprofit organisations.

Patient and public involvement: Patients and/or the public did not participate in the design, conduct, reporting, or dissemination plans for this study.

REFERENCES

1 Dixon MJ, Marazita ML, Beaty TH, et al. Cleft lip and palate: understanding genetic and environmental influences. *Nat Rev Genet* 2011;12:167–78.

2 Babai A, Irving M. Orofacial clefts: genetics of cleft lip and palate. *Genes (Basel)* 2023;14. doi: 10.3390/genes14081603 [Published online first: 2023/08/26]..

3 Leslie EJ, Marazita ML. Genetics of cleft lip and cleft palate. *Am J Med Genet C Semin Med Genet* 2013;163C:246–58. doi: 10.1002/ajmg.c.31381.

4 Kantar RS, Hamdan US, Muller JN, et al. Global prevalence and burden of orofacial clefts: A systematic analysis for the global burden of disease Study 2019. *J Craniofac Surg* 2023;34:2012–15. doi: 10.1097/SCS.0000000000009591 [Published online first: 2023/08/15]..

5 Zaaba MIS, Mokhtar KI, Rajion ZA. Revisiting genetics of cleft lip with or without cleft palate and cleft palate only: A narrative review. *Arch Orofac Sci* 2023;18:73–88. doi: 10.21315/aos2023.1802.RV01.

6 Mossey PA, Little J, Munger RG, et al. Cleft lip and palate. *Lancet* 2009;374:1773–85. doi: 10.1016/S0140-6736(09)60695-4 [Published online first: 2009/09/15]..

7 Kawalec A, Nelke K, Pawlas K, et al. Risk factors involved in orofacial cleft predisposition - review. *Open Med (Wars)* 2015;10:163–75. doi: 10.1515/med-2015-0027.

8 Huang H, Jiang J, Wang X, et al. Exposure to prescribed medication in early life and impacts on gut microbiota and disease development. *EClinicalmedicine* 2024;68:102428. doi: 10.1016/j.eclinm.2024.102428 [Published online first: 2024/02/05]..

9 Lin JM, Ding J, Di XM, et al. Association between prenatal antibiotics exposure and measures of fetal growth: A repeated-measure study. *Ecotoxicol Environ Saf* 2022;244:114041. 10.1016/j.ecoenv.2022.114041.

10 Choi A, Lee H, Jeong HE et al. Association between exposure to antibiotics during pregnancy or early infancy and risk of autism spectrum disorder, intellectual disorder, language disorder, and epilepsy in children: population based cohort study. *Br Med J* 2024;385:e076885. 10.1136/bmj-2023-076885.

11 Orwa SA, Gudnadottir U, Boven A, et al. Global prevalence of antibiotic consumption during pregnancy: A systematic review and meta-analysis. *J Infect* 2024;89:106189. doi: 10.1016/j.jinf.2024.106189 [Published online first: 2024/06/07]..

12 Bookstaver PB, Bland CM, Griffin B, et al. A review of antibiotic use in pregnancy. *Pharmacotherapy* 2015;35:1052–62. doi: 10.1002/phar.1649 [Published online first: 2015/11/26]..

13 Martinez de Tejada B. Antibiotic use and misuse during pregnancy and delivery: benefits and risks. *Int J Environ Res Public Health* 2014;11:7993–8009. doi: 10.3390/ijerph110807993 [Published online first: 2014/08/12]..

14 Tsamantioti ES, Hashmi MF. Teratogenic medications. StatPearls. In: StatPearls. Treasure Island (FL): StatPearls Publishing; January 10, 2024.

15 Stokes JM, Lopatkin AJ, Lobritz MA, et al. Bacterial metabolism and antibiotic efficacy. *Cell Metab* 2019;30:251–59. doi: 10.1016/j.cmet.2019.06.009 [Published online first: 2019/07/08]..

16 Ailes EC, Gilboa SM, Gill SK, et al. Association between antibiotic use among pregnant women with urinary tract infections in the first trimester and birth defects, national birth defects prevention study 1997 to 2011. *Birth Defects Res A Clin Mol Teratol* 2016;106:940–49. doi: 10.1002/bdra.23570.

17 Crider KS, Cleves MA, Reefhuis J, et al. Antibacterial medication use during pregnancy and risk of birth defects: national Birth Defects Prevention Study. *Arch Pediatr Adolesc Med* 2009;163:978–85. doi: 10.1001/archpediatrics.2009.188 [Published online first: 2009/11/04]..

18 Lin KJ, Mitchell AA, Yau WP, et al. Maternal exposure to amoxicillin and the risk of oral clefts. *Epidemiology* 2012;23:699–705. doi: 10.1097/EDE.0b013e318258cb05 [Published online first: 2012/07/07]..

19 Puhó EH, Szunyogh M, Métneki J, et al. Drug treatment during pregnancy and isolated orofacial clefts in hungary. *Cleft Palate Craniofac J* 2007;44:194–202. doi: 10.1597/05-208.1 [Published online first: 2007/03/03]..

20 Daniel S, Doron M, Fishman B, et al. The safety of amoxicillin and clavulanic acid use during the first trimester of pregnancy. *Br J Clin Pharmacol* 2019;85:2856–63. doi: 10.1111/bcp.14118 [Published online first: 2019/09/06]..

21 Damkier P, Brønniche LMS, Korch-Frandsen JFB, et al. In utero exposure to antibiotics and risk of congenital malformations: a population-based study. *Am J Obstet Gynecol*

2019;221:648.e1–648.e15. doi: 10.1016/j.ajog.2019.06.050 [Published online first: 2019/07/02]..

22 Mølgaard-Nielsen D, Hviid A. Maternal use of antibiotics and the risk of orofacial clefts: a nationwide cohort study. *Pharmacoepidemiol Drug Saf* 2012;21:246–53. doi: 10.1002/pds.2179 [Published online first: 2011/11/30]..

23 Leke AZ, Dolk H, Loane M, et al. Macrolide and lincosamide antibiotic exposure in the first trimester of pregnancy and risk of congenital anomaly: A European case-control study. *Reprod Toxicol* 2021;100:101–08. doi: 10.1016/j.reprotox.2021.01.006 [Published online first: 2021/01/18]..

24 Moher D, Shamseer L, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* 2015;4:1. doi: 10.1186/2046-4053-4-1 [Published online first: 2015/01/03]..

25 Shamseer L, Moher D, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ* 2015;350:g7647. doi: 10.1136/bmj.g7647 [Published online first: 2015/01/04]..

26 Ouzzani M, Hammady H, Fedorowicz Z, et al. Rayyan-a web and mobile app for systematic reviews. *Syst Rev* 2016;5:210.

27 Skrivankova VW, Richmond RC, Woolf BAR, et al. Strengthening the reporting of observational studies in epidemiology using Mendelian randomisation (STROBE-MR): explanation and elaboration. *Br Med J* 2021;375:n2233. 10.1136/bmj.n2233.

Table 1: Search strategy of databases

No	Database	Search strategy
1	PubMed	(("antibiotics" [MeSH Terms] OR "antimicrobial agents" [MeSH Terms] OR "anti-infective agents" [MeSH Terms] OR "antibacterial drugs" [All Fields] OR "broad spectrum antibiotics" [All Fields]) AND ("prenatal exposure delayed effects" [MeSH Terms] OR "maternal exposure" [All Fields] OR "intrauterine exposure" [All Fields] OR "gestational drug exposure" [All Fields]) AND ("orofacial clefts" [MeSH Terms] OR "cleft lip" [MeSH Terms] OR "cleft palate" [MeSH Terms] OR "congenital defects" [MeSH Terms] OR "birth defects" [All Fields] OR "congenital anomalies" [All Fields]) AND ("observational study" [MeSH Terms] OR "longitudinal study" [MeSH Terms] OR "retrospective study" [MeSH Terms] OR "prospective study" [MeSH Terms] OR "epidemiological research" [All Fields]))
2	Web of Science	TS=("antibiotics" OR "antimicrobial agents" OR "anti-infective agents" OR "antibacterial drugs" OR "broad spectrum antibiotics") AND TS=("prenatal exposure delayed effects" OR "maternal exposure" OR "intrauterine exposure" OR "gestational drug exposure") AND TS=("orofacial clefts" OR "cleft lip" OR "cleft palate" OR "congenital defects" OR "birth defects" OR "congenital anomalies") AND TS=("observational study" OR "longitudinal study" OR "retrospective study" OR "prospective study" OR "epidemiological research")

3	EMBASE	('antibiotic'/exp OR 'antimicrobial agent'/exp OR 'anti-infective agent'/exp OR 'antibacterial drug' OR 'broad spectrum antibiotic') AND ('prenatal exposure'/exp OR 'maternal exposure' OR 'intrauterine exposure' OR 'gestational drug exposure') AND ('orofacial cleft'/exp OR 'cleft lip'/exp OR 'cleft palate'/exp OR 'congenital defect'/exp OR 'birth defect' OR 'congenital anomaly') AND ('observational study'/exp OR 'longitudinal study'/exp OR 'retrospective study'/exp OR 'prospective study'/exp OR 'epidemiological research')
4	Scopus	(TITLE-ABS-KEY(antibiotics) OR TITLE-ABS-KEY(antimicrobial agents) OR TITLE-ABS-KEY(anti-infective agents) OR TITLE-ABS-KEY(antibacterial drugs) OR TITLE-ABS-KEY(broad spectrum antibiotics)) AND (TITLE-ABS-KEY(prenatal exposure delayed effects) OR TITLE-ABS-KEY(maternal exposure) OR TITLE-ABS-KEY(intrauterine exposure) OR TITLE-ABS-KEY(gestational drug exposure)) AND (TITLE-ABS-KEY(orofacial clefts) OR TITLE-ABS-KEY(cleft lip) OR TITLE-ABS-KEY(cleft palate) OR TITLE-ABS-KEY(congenital defects) OR TITLE-ABS-KEY(birth defects) OR TITLE-ABS-KEY(congenital anomalies)) AND (TITLE-ABS-KEY(observational study) OR TITLE-ABS-KEY(longitudinal study) OR TITLE-ABS-KEY(retrospective study) OR TITLE-ABS-KEY(prospective study) OR TITLE-ABS-KEY(epidemiological research))

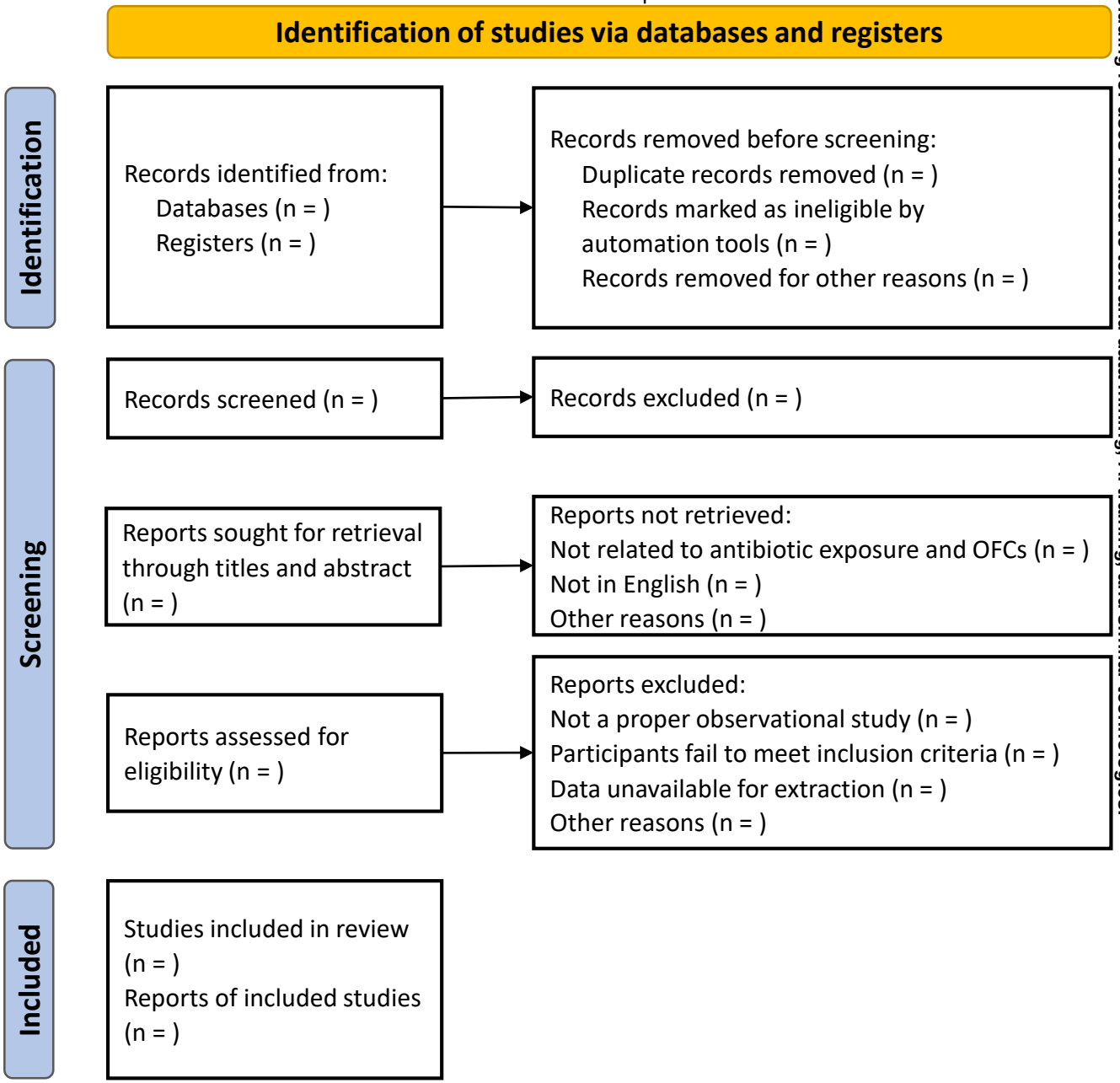
5	PSycINFO	("antibiotics" OR "antimicrobial agents" OR "anti-infective agents" OR "antibacterial drugs" OR "broad spectrum antibiotics") AND ("prenatal exposure delayed effects" OR "maternal exposure" OR "intrauterine exposure" OR "gestational drug exposure") AND ("orofacial clefts" OR "cleft lip" OR "cleft palate" OR "congenital defects" OR "birth defects" OR "congenital anomalies") AND ("observational study" OR "longitudinal study" OR "retrospective study" OR "prospective study" OR "epidemiological research")
6	Cochrane Library	("antibiotics" [MeSH descriptor] OR "antimicrobial agents" OR "anti-infective agents" OR "antibacterial drugs" OR "broad spectrum antibiotics") AND ("prenatal exposure delayed effects" [MeSH descriptor] OR "maternal exposure" OR "intrauterine exposure" OR "gestational drug exposure") AND ("orofacial clefts" [MeSH descriptor] OR "cleft lip" OR "cleft palate" OR "congenital defects" OR "birth defects" OR "congenital anomalies") AND ("observational studies" [MeSH descriptor] OR "longitudinal studies" OR "retrospective studies" OR "prospective studies" OR "epidemiological research")

366

367

Figure 1: Flowchart illustrating the study process, following the PRISMA-P guidelines.

PRISMA-P, Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols.



BMJ Open

Prenatal exposure to antibiotics and the risk of orofacial clefts: a protocol for a systematic review and meta-analysis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2024-092019.R1
Article Type:	Protocol
Date Submitted by the Author:	09-Oct-2024
Complete List of Authors:	Nagata, Abir; Osaka University Rahman, Md. Shafiur ; School of Health Innovation, Kanagawa University of Human Services; Research Centre for Child Mental Development, Hamamatsu University School of Medicine Rahman, Mahfuzur; Graduate School of Public Health, St. Luke's International University Nakagawa, Takatoshi; Graduate School of Medicine, Osaka University Sharmin, Salma; Department of Dental Public Health, University Dental College and Hospital Ohnishi, Kazunari ; Graduate School of Public Health, St. Luke's International University Rahman, Mahbubur; Graduate School of Public Health, St. Luke's International University
Primary Subject Heading:	Epidemiology
Secondary Subject Heading:	Epidemiology, Public health
Keywords:	Anti-Bacterial Agents, Fetal medicine < OBSTETRICS, EPIDEMIOLOGY, Child

TITLE

Prenatal exposure to antibiotics and the risk of orofacial clefts: a protocol for a systematic review and meta-analysis

Abir Nagata[1], MD; Shafiur Rahman[2,3], MD; Mahfuzur Rahman[4]; Takatoshi Nakagawa[1]; Salma Sharmin[5]; Kazunari Onishi[4]; and Mahbubur Rahman[4]

[1]Graduate School of Medicine, Osaka University, Osaka, Japan

[2]Research Centre for Child Mental Development, Hamamatsu University School of Medicine, Hamamatsu, Japan

[3]United Graduate School of Child Development, Osaka University, Kanazawa University, Hamamatsu University School of Medicine, Chiba University, and University of Fukui, Suita, Japan

[4]Graduate School of Public Health, St. Luke's International University, Tokyo, Japan

[5]Department of Dental Public Health, University Dental College and Hospital, Dhaka, Bangladesh

E-Mails:

Abir Nagata: abir.med@osaka-u.ac.jp ; MD Shafiur Rahman: srahman@hama-med.ac.jp ; MD Mahfuzur Rahman: mdmahfuzur08@gmail.com ; Takatoshi Nakagawa: tnakagawa@r-derma.med.osaka-u.ac.jp ; Salma Sharmin: sharminbd_0027@yahoo.com ; Kazunari Onishi: kaznaly@slcn.ac.jp ; Mahbubur Rahman: rahman@slcn.ac.jp

CORRESPONDENCE

Abir Nagata, MPH, PhD

Graduate School of Medicine, Osaka University, Osaka, Japan

E-mail: abir.med@osaka-u.ac.jp

Tel: (81) 6-6879-3960

Word count

Abstract: 348 words

Full text: 2670 words

Tables: 0

Figures: 1

Supplemental material: 2

ABSTRACT

Introduction: Orofacial clefts (OFCs), including cleft lip, cleft palate, and combined cleft lip and palate, are among the most common craniofacial malformations in newborns and present significant healthcare challenges. Emerging evidence has raised concerns regarding the potential impact of prenatal exposure to antibiotics on foetal development. Antibiotics prescribed during pregnancy—particularly those that cross the placental barrier—may pose teratogenic risks. Previous studies investigating the association between prenatal antibiotic exposure and the risk of OFCs have yielded inconsistent results. However, no studies have yet attempted to summarise this evidence, highlighting the need for a comprehensive evaluation. This report describes a systematic review and meta-analysis protocol to retrospectively analyse the relationship between prenatal antibiotic exposure and the risk of developing OFCs, focusing on the role of antibiotic type and timing of exposure. The results of such a review will hopefully provide a comprehensive synthesis of the available evidence, helping to inform clinical practice and guide patient counselling regarding the use of antibiotics during pregnancy.

Methods and analysis: The planned systematic review and meta-analysis will adhere to the PRISMA-P guidelines to ensure a comprehensive and systematic approach to summarising the available evidence on the topic. This study will include longitudinal cohort studies, case–control studies, and interventional trials that investigate the association between prenatal antibiotic exposure and OFCs. The search strategy will cover major databases, including CINAHL, Cochrane Library, ClinicalTrials.gov, EMBASE, PubMed, Scopus, and Web of Science, using tailored search terms. A team of independent assessors will screen article titles, abstracts, and full texts. Any discrepancies will be resolved through discussions. Quality assessment will utilise the Newcastle–Ottawa Scale and GRADE criteria. Data extraction will focus on the study

characteristics, participant details, exposure specifics, and outcome measures. A random-effects meta-analysis will aggregate summary effect sizes, and heterogeneity will be assessed using I^2 and Q statistics.

Ethics and dissemination: Ethical approval is not required for this systematic review, as it relies on already published data. The findings will be disseminated through peer-reviewed journals and conference presentations, providing critical insights into clinical practice and public health policies regarding antibiotic use during pregnancy.

PROSPERO registration number: CRD42024565064

Key words: antibiotics; orofacial clefts; cleft lip; cleft palate

STRENGTHS AND LIMITATIONS OF THE STUDY

- A comprehensive search strategy across multiple databases and grey literature sources will be employed while adhering to PRISMA-P guidelines, ensuring thorough identification of relevant studies.
- Study selection, data extraction, and quality assessments will be performed by two independent reviewers, enhancing accuracy and consistency.
- The use of established quality assessment tools, including the Newcastle–Ottawa Scale and GRADE criteria, will improve the reliability and validity of the findings.
- Variability in study designs, populations, antibiotic types, and timings of exposure may introduce heterogeneity, complicating the synthesis of results and resulting in publication bias.

INTRODUCTION

Orofacial clefts (OFCs) are among the most common craniofacial malformations in newborns and present significant healthcare challenges because of their complex aetiology and multifaceted impact on health.[1-3] They affect an estimated 4.6 million individuals globally, resulting in a burden of ~529,758.92 disability-adjusted life years.[4] OFCs—which include cleft lip (CL), cleft palate (CP), and combined cleft lip and palate (CLP)—result from disruptions in the normal development of the orofacial region during embryogenesis[2] and are specifically influenced by a combination of genetic, environmental, and maternal lifestyle factors.[2 3 5] Environmental influences, including maternal smoking, alcohol consumption, nutritional deficiencies, and medication use during pregnancy, have been implicated in the pathogenesis of OFCs.[5-7]

Recent research has raised concerns regarding the potential impact of prenatal exposure to antibiotics on foetal development.[8-10] Antibiotics are commonly prescribed during pregnancy to manage infections that, if left untreated, can pose significant risks to both the mother and the developing foetus.[11-13] However, the teratogenic potential of antibiotics, particularly those that cross the placental barrier, remains a topic of considerable debate and investigation.[14] Potential mechanisms through which antibiotics may contribute to the development of OFCs include the disruption of normal cellular processes, interference with folate metabolism, and induction of oxidative stress in the developing embryo.[15]

Several studies have suggested an association between prenatal antibiotic exposure and an increased risk of OFCs.[16-19] However, current evidence on the matter is inconsistent overall,[20-23] varying significantly across different antibiotic classes, dosages, and timings of exposure. Specifically, the current body of literature on this topic is characterized by heterogeneity in study

designs and populations, potentially due to variations in study populations, classifications of antibiotic exposure, and the control of confounding variables. Therefore, a systematic approach to synthesising this evidence is essential for drawing robust and generalisable conclusions. Understanding the potential teratogenic effects of antibiotics is crucial for clinical practice and public health. Therefore, the following proposed study is not only important for informing clinical practice and guiding patient counselling but also pivotal for shaping public health policies and future research trajectories in prenatal care and teratology.

OBJECTIVES

The objective is to answer the following PICO (Population, Intervention, Comparison, Outcome) question: what is the association between prenatal antibiotic exposure and the risk of developing OFCs in children, compared to the risk in children with no antibiotic exposure during pregnancy?

Specific objectives:

1. To systematically review studies investigating the relationship between prenatal antibiotic exposure and the risk of developing OFCs, with a particular focus on elucidating the role of antibiotic type, dosage, and timing of exposure.
2. To conduct a meta-analysis that quantitatively synthesises data from individual studies, providing a robust estimate of the association between prenatal antibiotic exposure and the risk of OFCs while accounting for potential moderating factors.

METHODS AND ANALYSIS

Protocol development

The protocol for this study aligns with the guidelines established by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P).[24 25] The PRISMA-Protocols checklist is provided in online supplemental file 1. The registration code for this review protocol (CRD42024565064) will be accessible on the PROSPERO International Prospective Register of Systematic Reviews. Any updates to the protocol and review process will be promptly reflected in the PROSPERO registration. The study is scheduled to commence in December 2024 and is expected to be completed by May 2025.

ELIGIBILITY

PICO Framework

Population: pregnant individuals and their children (mother–child pairs).

Intervention/Exposure: prenatal exposure to antibiotics.

Comparator: no exposure to antibiotics.

Outcome: the occurrence of OFCs, CL, CP, and CLP.

Inclusion and exclusion criteria

Participants

The participants in this study will be mother–child pairs. We will include all pregnant individuals regardless of age, ethnicity, or health status. We will include studies with explicit documentation of antibiotic exposure during pregnancy—including prescription records, patient self-reports, and medical notes confirming antibiotic use. The exclusion criteria include studies lacking specific information regarding the pregnancy status of the participants or those with ambiguous details regarding the timing and confirmation of pregnancy. We will also exclude studies in which

antibiotic exposure is not explicitly linked to the pregnancy period or where such exposure is inferred but not documented.

Exposure

Exposure to any class of antibiotics during pregnancy—including (but not limited to) penicillins, cephalosporins, macrolides, tetracyclines, fluoroquinolones, and sulfonamides—will be included. We will analyse antibiotic exposure by individual classes to better understand the potential differences in risks associated with each class. Given that certain classes of antibiotics are contraindicated during pregnancy,[12] this approach will allow for a more detailed analysis of class-specific effects. We will include studies that provide details regarding the type of antibiotic (specific name or class), frequency of intake, and trimester during which the exposure occurred. The primary focus will be on exposures occurring during the first trimester, as this is the period when orofacial cleft development occurs.[26] This detailed information is crucial for understanding the potential dose–response relationships and most critical periods of foetal development. Studies with grouped antibiotic exposures that do not distinguish between different antibiotics or classes will be excluded. This is due to the fact that different antibiotic classes may have distinct risks and mechanisms of action, and combining them may mask the specific effects associated with each individual class. Reports with unclear or inconsistent information regarding the frequency, dosage, or timing of antibiotic exposure during pregnancy will also be excluded.

Comparator/Control

Groups of pregnant individuals who did not receive any antibiotics during pregnancy will serve as a baseline control group for comparisons. In addition to the baseline control group, we will include active comparators, such as pregnant individuals who were prescribed antibiotics known

to be safe during pregnancy. This will help account for potential confounding by indication, as some bacterial infections themselves may increase the risk of birth defects.[27] Furthermore, we will consider comparator groups based on the timing of antibiotic exposure (e.g., first-trimester exposure vs. second and/or third-trimester exposure). Finally, we will exclude studies that lack a well-defined control or comparison group, thus failing to provide a clear contrast for the analysis.

Outcomes

The primary focus of this study will be on OFCs, including both syndromic and non-syndromic CL, CP, and combined CLP. All OFC diagnoses should be based on clinical examinations, medical records, or standardised screening protocols—either prenatally, at birth, or within a defined postnatal period (up to 1 year). Studies that focused on outcomes unrelated to OFCs or speculative studies, will be excluded.

Types of studies

Longitudinal cohort studies (both prospective and retrospective), case–control studies, and interventional trials that quantitatively assessed the relationship between prenatal antibiotic exposure and OFCs will be included. All included studies must have a sound methodological design, including well-defined populations, clear exposure and outcome measures, and appropriate statistical analyses. Cross-sectional studies will be excluded as they do not establish a temporal relationship between exposure and outcome; therefore, causality cannot be inferred. We will also exclude case reports and case series, as they often lack generalisability, as well as reviews, editorials, commentaries, and animal studies, because they typically do not provide original empirical data. Studies with methodological flaws, such as inadequate sample sizes or lack of statistical rigor, will also be excluded. No restrictions will be applied regarding the

language of the studies or publication length, and translation will be performed using Google Translate or by individuals capable of providing a translation.

Search strategy and study selection

Our search methodology has been meticulously designed to identify both published and unpublished studies. This comprehensive approach covers electronic repositories, conference records, virtual platforms, scholarly dissertations, and (if necessary) direct correspondences with primary authors. We will search databases including CINAHL, Cochrane Library, ClinicalTrials.gov, EMBASE, PubMed, Scopus, and Web of Science. Index terms and keywords will be carefully tailored to the unique characteristics of each database. Google Scholar will also be used to search for gray literature and ongoing studies. The following search terms will be combined and adapted as needed to meet the database specifications: ("antibiotics" OR "antimicrobial agents" OR "anti-infective agents" OR "antibacterial drugs" OR "broad spectrum antibiotics" AND "prenatal exposure delayed effects" OR "maternal exposure" OR "intrauterine exposure" OR "gestational drug exposure") AND ("orofacial clefts" OR "cleft lip" OR "cleft palate" OR "congenital defects" OR "birth defects" OR "congenital anomalies" AND ("observational study" OR "longitudinal study" OR "retrospective study" OR "prospective study" OR "epidemiological research". Both MeSH terms and other appropriate subject headings will be used in the database search. The search strategy for the databases is illustrated in online supplemental file 2.

A team of three independent assessors will conduct the search. The retrieved studies will be imported into Rayyan (https://rayyan.ai/), a systematic review software platform[28] Initially, two independent assessors will assess the suitability of the titles and abstracts of all screened publications, followed by full-text examinations in accordance with our inclusion criteria. Two

assessors will concurrently evaluate the abstracts and full-text materials, and any discrepancies will be resolved through comprehensive discussions. **Figure 1** illustrates the screening process. In cases where significant disparities persist, the ultimate resolution will be entrusted to the other assessors.

Quality assessment

Two assessors will independently evaluate the methodological rigor of the included studies using the Newcastle–Ottawa Scale to assess the quality of the non-randomised studies included in the meta-analyses. If RCTs are included, the Cochrane Risk of Bias (ROB V.2)[29] tool will be applied. Additionally, two assessors will independently apply the GRADE assessment to categorise the quality and strength of evidence in each publication as high, moderate, low, or very low in terms of its primary outcomes. Any discrepancies between assessments will be resolved through constructive discussions. The assessment of methodological quality will be integrated into the discussion of the respective study findings.

Data extraction

Two assessors will independently review the included studies and extract relevant information using a pre-designed form. Discrepancies will be resolved through discussion until a consensus is reached. The data extracted from each study will encompass, though not be limited to: (a) *study characteristics*: author names, publication year, citation, study location, study design, study dates, participant selection criteria, statistical analysis methods, funding sources, and conflicts of interest; (b) *participant characteristics*: including the number of mother–child pairs (sample size), sampling methods, sex, age, and any reported sociodemographic data; (c) *exposure*: type of antibiotic (specific name or class), frequency of use, the trimester during which exposure

occurred, and the method of data collection; (d) *comparator*: participants who were not exposed to antibiotics; (e) *outcome*: type of OFCs, such as CL, CP, or combined CLP, the method of outcome data collection, and covariate adjustment in the analysis; and (f) *results*: statistical outcomes, including effect measures, confidence intervals, and p-values.

OFC diagnoses must be confirmed through clinical examinations, medical records, or standardised screening protocols, either during the prenatal stage, at birth, or within a specified postnatal period. Secondary outcomes may include variations in the risk of OFCs based on antibiotic dosage, active comparators, and socio-economic status. We will prioritise studies with the clearest and most consistent definitions of both exposure and outcomes.

Data analysis and synthesis

The findings will be reported and presented in accordance with the PRISMA statement.[30] Additionally, if only observational studies are included, we will also follow the MOOSE (Meta-Analysis of Observational Studies in Epidemiology) checklist.[31] The findings will be comprehensively presented using tables and figures. The effect size (ES) of interest will be the relative risk (RR). When effect estimates are provided as crude or adjusted odds ratios (ORs) or RRs, we will prioritise collecting the adjusted estimates and provide a descriptive summary of the covariates adjusted for each study. In cases where the ES is not present, an unadjusted RR will be estimated from contingency tables. ORs will be converted to RRs using appropriate formulae. Both fixed-effect and random-effects meta-analyses will be used to estimate the pooled ESs along with their associated 95% confidence intervals. Significance levels will be documented. Inter-study variability, measured using the Cochrane Q or I^2 statistics, will be explored, as well as potential impacts from smaller studies. I^2 values of 25%, 50%, and 75% will be assumed to represent low, moderate, and high heterogeneity, respectively. The

significance of heterogeneity will be determined via χ^2 values for Q statistics, with p<0.05. If the level of between-study heterogeneity is higher (I^2 > 75%), a random-effects meta-regression will be performed to identify the potential moderators. A leave-one-sample-out validation will be used to explore the influences of each included study on the pooled ES. Funnel plots will be used to detect potential publication bias and small-study effects. p<0.05 will be considered indicative of statistically significant publication bias. For outcomes with more than 10 individual studies, we will use Egger's regression test to assess asymmetry. The combined effect size will be visually represented using a forest plot. If the number of studies included is insufficient (<3), a narrative review of the study findings will be presented instead of a meta-analysis.

Sensitivity analyses on primary outcomes will be conducted and will involve excluding studies with a high risk of bias or incomplete data. Efforts will be made to contact researchers or study sponsors to obtain any missing information. If this is not possible, established methods for estimating missing data using multiple imputations will be applied. The validity of imputed data will be assessed through a sensitivity analysis. Subgroup analyses will explore the effects of medication dosages and active comparators. Furthermore, we will conduct a meta-regression analysis across various subgroups, including, but not limited to, confounding factors (crude or adjusted), socio-economic status, and study design. All statistical analyses will be conducted in Stata version 18 (StataCorp).

DISCUSSION

This systematic review and meta-analysis protocol represents the first comprehensive effort to investigate the association between prenatal antibiotic exposure and the risk of OFCs. Its findings are expected to provide critical insights into medication safety during pregnancy and its potential teratogenic effects on foetal development, particularly regarding the occurrence of

OFCs. Our conclusions will be based on the combined results of the included studies, presented through quantitative analysis or narrative synthesis.

Understanding the relationship between prenatal antibiotic exposure and OFCs is crucial for clinical decision-making and patient counselling. If a significant association is found, it will highlight the need for careful consideration of antibiotic prescriptions during pregnancy. This could lead to the development of guidelines and protocols aimed at minimising unnecessary antibiotic use and selecting safer alternatives when treatment is necessary. The results will also hopefully ensure that healthcare providers are better equipped to inform expectant mothers about the potential risks and benefits of antibiotic use during pregnancy, thereby facilitating more informed choices.

The major strengths of this planned, systematic review lie in its rigorous methodological approach, adherence to the PRISMA-P guidelines, and use of established quality assessment tools such as the Newcastle–Ottawa Scale and GRADE criteria. However, several methodological challenges must also be addressed. First, the included studies are likely to vary in their designs, populations, antibiotic types, dosages, and timing of exposure, which may introduce significant heterogeneity. This variability can complicate the synthesis of the results and limit the generalisability of our findings. Subgroup and sensitivity analyses will be crucial to addressing these issues. Second, the potential under-representation of unpublished or negative findings may also introduce publication bias. Funnel plots and Egger's tests will be used to assess and mitigate this bias; however, its presence cannot be entirely ruled out. Third, the conclusions drawn from this review will depend on the methodological quality and reporting standards of the included studies. Variations regarding how antibiotic exposure and OFCs are defined and measured may impact the robustness of the findings. The use of the GRADE criteria

will help evaluate the strength and quality of the evidence, providing a clearer understanding of the confidence that can be placed in the results. Nevertheless, this review is expected to identify gaps in the current literature and suggest areas for future research.

ETHICS AND DISSEMINATION

Obtaining ethical approval was not deemed necessary for this study, as it pertains to a protocol for a systematic review that relies on published, and therefore, publically available data. The findings of this study will be communicated through peer-reviewed articles and presentations at conferences.

ADDITIONAL INFORMATION

Author contributions: AN, MSF, and MMR conceived of the study's concept and drafted the initial version. TK, SS, KO, and MR provided guidance to the research teams. All of the authors participated in drafting and revising the manuscript, formulating the review questions, and designing the study. All of the authors have read and approved the final version of this manuscript. AN serves as the guarantor, accepting full responsibility for the work and/or the conduct of the study, has access to the data, and controls the decision to publish.

Conflict of Interest Disclosures: The authors declare no competing interests.

Funding/Support: This research did not receive any specific funding from public, commercial, or nonprofit organisations.

Patient and public involvement: Patients and/or the public did not participate in the design, conduct, reporting, or dissemination plans for this study.

REFERENCES

1. Dixon MJ, Marazita ML, Beaty TH, et al. Cleft lip and palate: understanding genetic and environmental influences. Nat Rev Genet 2011;12(3):167-78

2. Babai A, Irving M. Orofacial clefts: genetics of cleft lip and palate. Genes (Basel) 2023;14(8). doi: 10.3390/genes14081603

3. Leslie EJ, Marazita ML. Genetics of cleft lip and cleft palate. Am J Med Genet C Semin Med Genet 2013;163C(4):246-58. doi: 10.1002/ajmg.c.31381

4. Kantar RS, Hamdan US, Muller JN, et al. Global prevalence and burden of orofacial clefts: A systematic analysis for the global burden of disease Study 2019. J Craniofac Surg 2023;34(7):2012-15. doi: 10.1097/SCS.0000000000009591

5. Zaaba MIS, Mokhtar KI, Rajion ZA. Revisiting genetics of cleft lip with or without cleft palate and cleft palate only: A narrative review. Arch Orofac Sci 2023;18(2):73-88. doi: 10.21315/aos2023.1802.RV01

6. Mossey PA, Little J, Munger RG, et al. Cleft lip and palate. Lancet 2009;374(9703):1773-85. doi: 10.1016/S0140-6736(09)60695-4 [published online first: 2009/09/15]

7. Kawalec A, Nelke K, Pawlas K, et al. Risk factors involved in orofacial cleft predisposition - review. Open Med (Wars) 2015;10(1):163-75. doi: 10.1515/med-2015-0027

8. Huang H, Jiang J, Wang X, et al. Exposure to prescribed medication in early life and impacts on gut microbiota and disease development. EClinicalmedicine 2024;68:102428. doi: 10.1016/j.eclinm.2024.102428

9. Lin JM, Ding J, Di XM, et al. Association between prenatal antibiotics exposure and measures of fetal growth: A repeated-measure study. Ecotoxicol Environ Saf 2022;244:114041. doi: 10.1016/j.ecoenv.2022.114041

10. Choi A, Lee H, Jeong HE et al.. Association between exposure to antibiotics during pregnancy or early infancy and risk of autism spectrum disorder, intellectual disorder, language disorder, and epilepsy in children: population based cohort study. Br Med J 2024;385:e076885. doi: 10.1136/bmj-2023-076885

11. Orwa SA, Gudnadottir U, Boven A, et al. Global prevalence of antibiotic consumption during pregnancy: A systematic review and meta-analysis. J Infect 2024;89(2):106189. doi: 10.1016/j.jinf.2024.106189

12. Bookstaver PB, Bland CM, Griffin B, et al. A review of antibiotic use in pregnancy. Pharmacotherapy 2015;35(11):1052-62. doi: 10.1002/phar.1649

13. Martinez de Tejada B. Antibiotic use and misuse during pregnancy and delivery: benefits and risks. Int J Environ Res Public Health 2014;11(8):7993-8009. doi: 10.3390/ijerph110807993

14. Tsamantioti ES, Hashmi MF. Teratogenic medications. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2024.

15. Stokes JM, Lopatkin AJ, Lobritz MA, et al.. Bacterial metabolism and antibiotic efficacy. Cell Metab 2019;30(2):251-59. doi: 10.1016/j.cmet.2019.06.009

16. Ailes EC, Gilboa SM, Gill SK, et al. Association between antibiotic use among pregnant women with urinary tract infections in the first trimester and birth defects, national birth defects

prevention study 1997 to 2011. Birth Defects Res A Clin Mol Teratol 2016;106(11):940-49. doi: 10.1002/bdra.23570

17. Crider KS, Cleves MA, Reefhuis J, et al. Antibacterial medication use during pregnancy and risk of birth defects: national Birth Defects Prevention Study. Arch Pediatr Adolesc Med 2009;163(11):978-85. doi: 10.1001/archpediatrics.2009.188

18. Lin KJ, Mitchell AA, Yau WP, et al. Maternal exposure to amoxicillin and the risk of oral clefts. Epidemiology 2012;23(5):699-705. doi: 10.1097/EDE.0b013e318258cb05

19. Puhó EH, Szunyogh M, Métneki J, et al. Drug treatment during pregnancy and isolated orofacial clefts in Hungary. Cleft Palate Craniofac J 2007;44(2):194-202. doi: 10.1597/05-208.1

20. Daniel S, Doron M, Fishman B, et al. The safety of amoxicillin and clavulanic acid use during the first trimester of pregnancy. Br J Clin Pharmacol 2019;85(12):2856-63. doi: 10.1111/bcp.14118

21. Damkier P, Brønniche LMS, Korch-Frandsen JFB, et al. In utero exposure to antibiotics and risk of congenital malformations: a population-based study. Am J Obstet Gynecol 2019;221(6):648.e1-648.e15. doi: 10.1016/j.ajog.2019.06.050

22. Mølgaard-Nielsen D, Hviid A. Maternal use of antibiotics and the risk of orofacial clefts: a nationwide cohort study. Pharmacoepidemiol Drug Saf 2012;21(3):246-53. doi: 10.1002/pds.2179 [published online first: 2011/11/30]

23. Leke AZ, Dolk H, Loane M, et al. Macrolide and lincosamide antibiotic exposure in the first trimester of pregnancy and risk of congenital anomaly: A European case-control study. Reprod Toxicol 2021;100:101-08. doi: 10.1016/j.reprotox.2021.01.006

24. Moher D, Shamseer L, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. Syst Rev 2015;4(1):1. doi: 10.1186/2046-4053-4-1

25. Shamseer L, Moher D, Clarke M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ 2015;350:g7647. doi: 10.1136/bmj.g7647

26. Kini U. Genetics and orofacial clefts: a clinical perspective. Br Dent J 2023;234(12):947-52. doi: 10.1038/s41415-023-5994-3

27. Kumar M, Saadaoui M, Al Khodor S. Infections and pregnancy: effects on maternal and child health. Front Cell Infect Microbiol 2022;12:873253. doi: 10.3389/fcimb.2022.873253

28. Ouzzani M, Hammady H, Fedorowicz Z, et al. Rayyan-a web and mobile app for systematic reviews. Syst Rev 2016;5(1):210

29. Sterne JAC, Savović J, Page MJ, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. Br Med J 2019;366:l4898. doi: 10.1136/bmj.l4898

30. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Br Med J 2021;134:178-89. doi: 10.1016/j.jclinepi.2021.03.001

31. Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. -Anal Obs Stud Epidemiol (Moose) Group JAMA 2000;283(15):2008-12. doi: 10.1001/jama.283.15.2008

Figure 1: Flowchart illustrating the study process, following the PRISMA-P guidelines.

PRISMA-P, Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols.

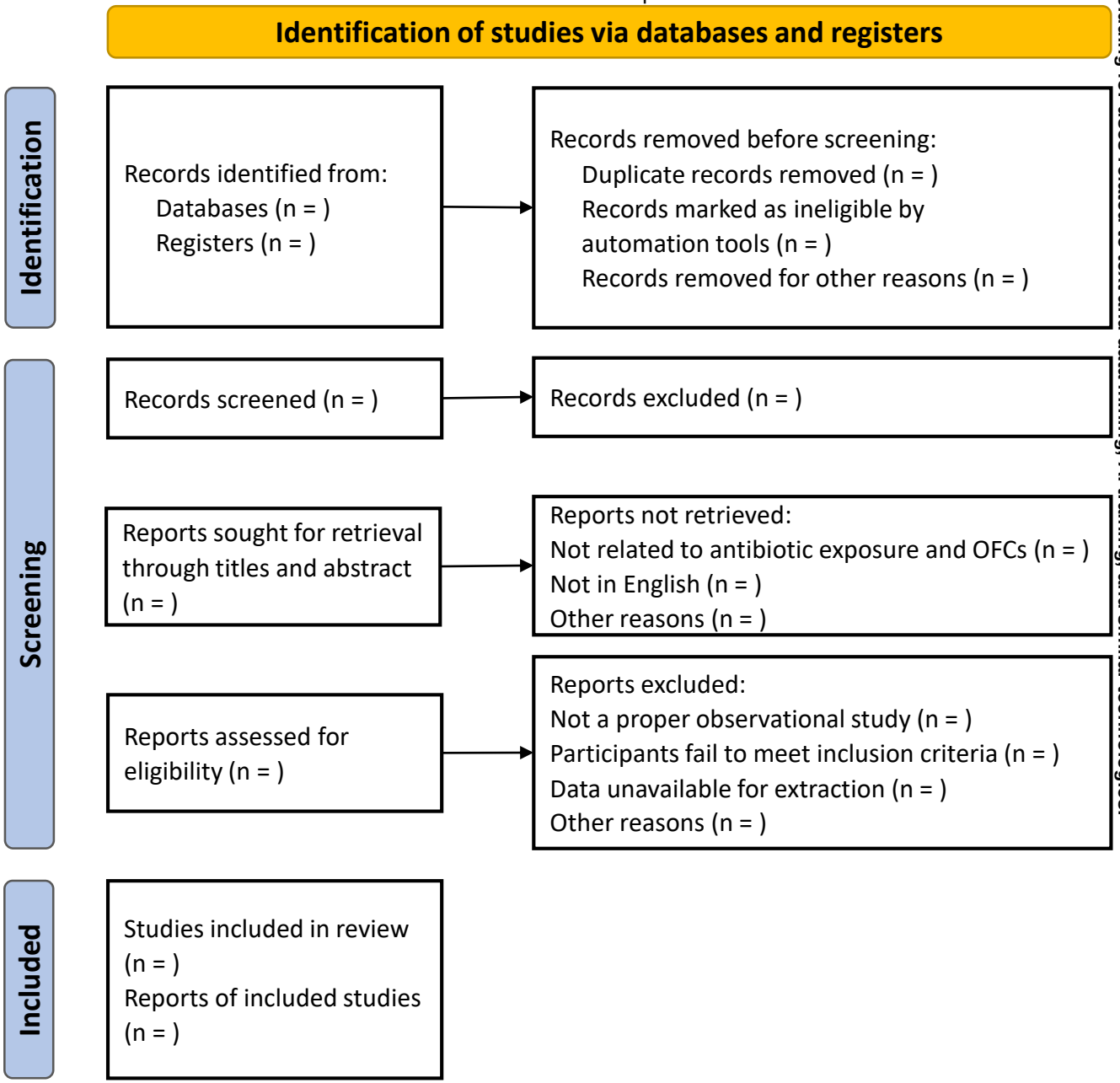


Table 1: Search strategy of databases

No.	Database	Search strategy
1	PubMed	(("antibiotics" [MeSH Terms] OR "antimicrobial agents" [MeSH Terms] OR "anti-infective agents" [MeSH Terms] OR "antibacterial drugs" [All Fields] OR "broad spectrum antibiotics" [All Fields]) AND ("prenatal exposure delayed effects" [MeSH Terms] OR "maternal exposure" [All Fields] OR "intrauterine exposure" [All Fields] OR "gestational drug exposure" [All Fields]) AND ("orofacial clefts" [MeSH Terms] OR "cleft lip" [MeSH Terms] OR "cleft palate" [MeSH Terms] OR "congenital defects" [MeSH Terms] OR "birth defects" [All Fields] OR "congenital anomalies" [All Fields]) AND ("observational study" [MeSH Terms] OR "longitudinal study" [MeSH Terms] OR "retrospective study" [MeSH Terms] OR "prospective study" [MeSH Terms] OR "epidemiological research" [All Fields] OR "randomized controlled trial" [MeSH Terms] OR "RCT" [All Fields] OR "clinical trial" [MeSH Terms]))
2	Web of Science	TS=("antibiotics" OR "antimicrobial agents" OR "anti-infective agents" OR "antibacterial drugs" OR "broad spectrum antibiotics") AND TS=("prenatal exposure delayed effects" OR "maternal exposure" OR "intrauterine exposure" OR "gestational drug exposure") AND TS=("orofacial clefts" OR "cleft lip" OR "cleft palate" OR "congenital defects" OR "birth defects" OR "congenital anomalies") AND TS=("observational study" OR "longitudinal study" OR "retrospective study" OR "prospective study" OR "epidemiological research" OR "randomized controlled trial" OR "RCT" OR "clinical trial")
3	EMBASE	('antibiotic'/exp OR 'antimicrobial agent'/exp OR 'anti-infective agent'/exp OR 'antibacterial drug' OR 'broad spectrum antibiotic') AND ('prenatal exposure'/exp OR 'maternal exposure' OR 'intrauterine exposure' OR 'gestational drug exposure') AND ('orofacial cleft'/exp OR 'cleft lip'/exp OR 'cleft palate'/exp OR 'congenital defect'/exp OR 'birth defect' OR 'congenital anomaly')

		AND ('observational study'/exp OR 'longitudinal study'/exp OR 'retrospective study'/exp OR 'prospective study'/exp OR 'randomized controlled trial'/exp OR 'RCT'/exp OR 'clinical trial'/exp)
4	Scopus	(TITLE-ABS-KEY(antibiotics) OR TITLE-ABS-KEY(antimicrobial agents) OR TITLE-ABS-KEY(anti-infective agents) OR TITLE-ABS-KEY(antibacterial drugs) OR TITLE-ABS-KEY(broad spectrum antibiotics)) AND (TITLE-ABS-KEY(prenatal exposure delayed effects) OR TITLE-ABS-KEY(maternal exposure) OR TITLE-ABS-KEY(intrauterine exposure) OR TITLE-ABS-KEY(gestational drug exposure)) AND (TITLE-ABS-KEY(orofacial clefts) OR TITLE-ABS-KEY(cleft lip) OR TITLE-ABS-KEY(cleft palate) OR TITLE-ABS-KEY(congenital defects) OR TITLE-ABS-KEY(birth defects) OR TITLE-ABS-KEY(congenital anomalies)) AND (TITLE-ABS-KEY(observational study) OR TITLE-ABS-KEY(longitudinal study) OR TITLE-ABS-KEY(retrospective study) OR TITLE-ABS-KEY(prospective study) OR TITLE-ABS-KEY(randomized controlled trial) OR TITLE-ABS-KEY(RCT) OR TITLE-ABS-KEY(clinical trial))
5	PSycINFO	("antibiotics" OR "antimicrobial agents" OR "anti-infective agents" OR "antibacterial drugs" OR "broad spectrum antibiotics") AND ("prenatal exposure delayed effects" OR "maternal exposure" OR "intrauterine exposure" OR "gestational drug exposure") AND ("orofacial clefts" OR "cleft lip" OR "cleft palate" OR "congenital defects" OR "birth defects" OR "congenital anomalies") AND ("observational study" OR "longitudinal study" OR "retrospective study" OR "prospective study" OR "randomized controlled trial" OR "RCT" OR "clinical trial")
6	Cochrane Library	("antibiotics" [MeSH descriptor] OR "antimicrobial agents" OR "anti-infective agents" OR "antibacterial drugs" OR "broad spectrum antibiotics")

		AND ("prenatal exposure delayed effects" [MeSH descriptor] OR "maternal exposure" OR "intrauterine exposure" OR "gestational drug exposure") AND ("orofacial clefts" [MeSH descriptor] OR "cleft lip" OR "cleft palate" OR "congenital defects" OR "birth defects" OR "congenital anomalies") AND ("observational studies" [MeSH descriptor] OR "longitudinal studies" OR "retrospective studies" OR "prospective studies" OR "randomized controlled trial" OR "RCT" OR "clinical trial")
7	ClinicalTrials.gov	Condition: "Orofacial clefts" OR "cleft lip" OR "cleft palate" OR "congenital defects" Intervention: "antibiotics" OR "antimicrobial agents" OR "anti-infective agents" OR "antibacterial drugs" Study Type: Interventional Studies (Clinical Trials)