Prenatal Exposure to Macrolides and Risk of Congenital Malformations: A Meta-Analysis

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Abstract

Introduction: Macrolides are widely used during pregnancy; however, their fetal safety remains uncertain. We performed a meta-analysis to assess the relation between prenatal exposure to macrolides and occurrence of congenital malformations.

Methods: We searched MEDLINE, EMBASE and other databases until June 12th, 2019. We assessed the quality of the studies and checked for heterogeneity and publication bias. We performed 3 different analyses and compared the effect of macrolides to each of the following unexposed populations: Group 1: babies unexposed to any medicine before birth, Group 2: babies exposed to non-macrolides antibiotics/non-teratogens, and Group 3: mixed population of the first and second comparators.

Results: A weak association between macrolides and congenital malformation of any type was observed when macrolides were compared to the mixed population [$OR_{group\ 3}$: 1.06 (95%CI 1.01, 1.10)]. Subgroup analysis showed that this weak association is restricted to fetus exposure in the first trimester of pregnancy [OR: 1.06 (95%CI: 1.01, 1.11)] and to cohort studies [OR: 1.07 (95%CI: 1.02, 1.13). Digestive system malformations were found to be slightly associated with prenatal exposure to macrolides [$OR_{group\ 3}$: 1.14 (95%CI: 1.02, 1.26)]. Musculoskeletal system was also found to be potentially affected [$OR_{group\ 2}$: 1.21 (95%CI: 1.08, 1.35) and $OR_{group\ 3}$: 1.15 (95%CI: 1.05, 1.26)]. European studies showed a slightly stronger association than American studies in these two comparisons.

Conclusions: Our study suggests a weak association of macrolides' prenatal use and congenital malformations, limited to exposure in early pregnancy, and musculoskeletal and digestive systems. In addition to studies with a larger control of confounding, risk-benefit research is needed to determine the usefulness of macrolides during pregnancy.

Key words: macrolides, congenital malformations, meta-analysis, fetal safety.

Key Points

- Macrolides are widely used during pregnancy, however knowledge about their fetal safety is uncertain.
- This meta-analysis shows that macrolides intake during pregnancy is associated with a weak increase in the odds of congenital abnormalities, limited to some subgroups.
- These findings along with additional assessment of the risks and benefits of macrolides are crucial to determine their usefulness during pregnancy.

1. Introduction

Congenital abnormalities are malformations of organs or body parts during organogenesis, which mainly take place in the first trimester of pregnancy [1,2]. Some malformations may also occur in the second and third trimesters of pregnancy as the tissues and organs continue to develop [2].

Birth defects are the leading risk factor of infants' mortality worldwide [1, 3-5]. However the causes of the occurrence of these defects are not well determined [1]. According to the Global Report on Birth Defects, the proportion of malformations due to genetic factors is small compared to the proportion of abnormalities due to exposure to teratogenic intra-uterine factors such as certain medicines [6]. It is remarkable that 97.7% of the drugs approved by the FDA between 2000 and 2010 have ''undetermined'' teratogenic risk in human pregnancy [7]. Antibiotics are frequently prescribed during pregnancy, mainly to treat urinary infections. Indeed, around one-fourth of pregnant women receive antibiotics during pregnancy, comprising thereby 80% of all prescriptions [8]. Some antibiotics used for this purpose were found to harm fetal formation [9].

Macrolides are among the most consumed antibacterial medicines [10-12]. The major types of macrolides include erythromycin, azithromycin, clarithromycin and roxithromycin. Data about the association of prenatal exposure to macrolides with birth defects are inconclusive [9, 13-16]. For instance, when used in early pregnancy, erythromycin was associated with anencephaly, transverse limb deficiency, pyloric stenosis and other congenital malformations, [9, 13] while no relation was found between clarithromycin and the risk of fetal malformations [16].

Except for a meta-analysis aimed at assessing general adverse child outcomes, which included a limited number of original studies, no comprehensive review was carried out on this topic so far [17]. Therefore, to determine if there is an effect of macrolides´ prenatal

exposure on congenital abnormalities and to study whether this effect varies according to the type of macrolide and to the exposure pregnancy term, we carried out a systematic review and meta-analysis.

2. Methods

2.1. Information sources and search strategy

We retrieved published studies on the use of macrolide antibiotics during pregnancy and the development of congenital malformations, by searching MEDLINE from 1966 until June 12th, 2019. To identify the relevant articles, we used the following syntax:

(macrolide* OR erythromycin OR roxithromycin OR clarithromycin OR azithromycin OR macrolide[MeSH Terms]) AND (((birth defect*) OR (congenital) OR "congenital abnormalities"[MeSH Terms] OR fetal OR fetus))). The search was exclusive to studies involving humans. Our search was not limited to any language of publication.

We excluded cross sectional studies from our search due to the impossibility of this design to infer any causal effect. We also conducted a search using the following terms as free text words: birth defects, congenital malformation, congenital abnormalities, pregnancy, macrolides, cohort, case-control and incidence. We adopted similar strategies to search EMBASE from 1980 until 2019; the five regional bibliographic databases of the World Health Organization (WHO): African Index Medicus (AIM), Latin American and Caribbean Health Science Literature Database (LILACS), Index Medicus for the Eastern Mediterranean Region (IMEMR), Index Medicus for South-East Asia Region (IMSEAR), Western Pacific Region Index Medicus (WPRIM); as well as the Open Access Thesis and Dissertations (OATD). We also searched for abstracts of scientific meetings using the Conference Proceedings Citation Index from inception in 1990 until June 2019. Finally, we manually examined the references of all obtained articles as well as those of related systematic reviews.

All searches were carried out independently by two epidemiologists (N.M. and B.T.) and the results were merged.

We registered the review protocol of this study in the International prospective register of systematic reviews PROSPERO (Reference: CRD42017055131) [18].

2.2. Eligibility criteria and study selection

We included studies that fulfilled the following eligibility criteria: (1) reporting original data from randomized clinical trials, case-control or cohort studies; (2) examining the association between prenatal exposure to macrolides and the development of congenital malformation; (3) providing estimates of relative risk (RR) or odds ratios (OR) and their corresponding 95% confidence intervals (CIs) or presenting enough data to calculate them. Due to their limitation in inferring causal relationships, cross-sectional studies were excluded. We restricted our analysis to congenital malformations in live births only. We excluded articles on drugs that could be assimilated to macrolides such as ivermectin, nystatin and natamycin and limited our study to true macrolides. We also excluded studies that investigated the postnatal maternal and/or infant exposure to macrolides. Duplicate studies were detected by identifying the study population. Only the most updated study was included in the meta-analysis. In addition, for publications about different macrolides that were carried out by the same investigator in the same population, we calculated the pooled RRs or ORs of these different publications and presented them as a single study. When effect measures of maternal exposure to different types of macrolides and/or during distinct terms of pregnancy were reported in the same study, we analyzed each outcome separately.

2.3. Data extraction

We scanned the titles and abstracts of the collected articles in order to exclude the irrelevant ones, and subsequently reviewed the full texts of the remaining articles to check their

eligibility. We recorded the following information from the eligible studies: (1) study name and source; (2) publication year; (3) study design (cohort study and case-control study); (4) study period; (5) sample size (number of cases and controls for case-control studies, or number of cases and cohort size for cohort studies); (6) type of control (unexposed to any drug, exposed to non-macrolides antibiotics or to non-teratogens, or a mixture of the unexposed and non-macrolide exposed fetuses), (7) study country; (8) ascertainment of macrolides exposure; (9) exposure dose; (10) exposure period; (11) effect measures and 95% confidence interval; (12) adjustment, matching, and restriction variables; (13) percentage of drop-outs in cohort studies; (14) response rate in case-control studies; (15) type of macrolide; and (16) use of individual or a mixture of macrolides.

2.4. Quality assessment

We assessed the quality of the studies by using a seven-point scale extracted from the Newcastle Ottawa scale according to the requirements of this meta-analysis [19]. We assessed the following criteria: Macrolide exposure ascertainment: based on a clinical history or any other documented proof (1 point), else (0 points). Confounding assessment: results adjusted for maternal age and urinary tract infections (2 points, 1 point each), else (0 points). Exposure description: reported duration (1 point) and determined dose (1 point), else (0 points). To assess methodological issues that were not common to cohort studies and case-control studies, we used the following criteria: drop-out rate or losses to follow up in cohort studies: < 20% (2 points), between 20% and 40% (1 point), and > 40% or not explained (0 points) and participation rate in case-control studies: > 80% (1 point), < 80% or not reported (0 points). We carried out a pooled analysis on studies scoring more than 4 points and compared the results with those of studies with a lower quality score.

Both data extraction process and quality rating were independently performed by two epidemiologists [N.M. and H.T.] and discrepancies were resolved through discussion with a third party [B.T.].

2.5. Data synthesis

We extracted the adjusted odds ratios and their 95% CI from the studies included in our meta-analysis. We used the crude ORs if no adjusted estimate was provided or computed them from the data provided by the authors. Subsequently, we weighted the log RRs and log OR for cohort and case-control studies, respectively, by the inverse of their variance to obtain a pooled OR and its 95% CI. Odds Ratios were considered unbiased estimates of the Relative Risk [20].

We carried out three different analyses according to the characteristics of the unexposed population. In the first approach, we included all studies that used fetuses unexposed to any drug as a comparator (comparison group 1). The second approach encompassed studies that compared the effect of macrolides parental exposure to exposure to non-macrolides or nonteratogenic drugs (comparison group 2). The third analysis involved the combination of the studies of the two approaches described above (comparison group 3).

We presented both fixed and random effects pooled estimates. We checked for heterogeneity using DerSimonian and Laird's Q test. We quantified the heterogeneity by calculating the proportion of the total variance due to between study variance (Ri) [21]. Large values (>0.75) of Ri indicate large amount of heterogeneity, values between 0.4 and 0.75 suggest a moderate amount while small values (<0.4) indicate low heterogeneity. Subsequently, we restricted the analysis to subgroups defined by study characteristics such as adjustment factors, exposure period, anatomical location of congenital malformations and study design. Prior to data analysis, we contacted the authors in order to know whether abstracts retrieved in our search

were published later as a full paper and to have more information about the comparison group used in the studies [14, 16, 22-25]. We repeated the same analysis for the three categories of comparison populations.

2.6. Assessment of publication bias

We assessed publication bias visually using funnel plots at first, and then, more formally, using Egger´s regression test [26]. Furthermore, we used the trim-and-fill method to correct for potential publication bias.

We also performed a sensitivity analysis by assuming that the results of case-control studies are the least likely to be published when their results show no effect. Accordingly, we recalculated the pooled OR assuming that (1) the case-control studies retrieved in our search represent only half of the studies ever conducted, (2) the unpublished studies found a null association (OR = 1) between macrolides prenatal exposure and congenital malformation, and (3) the unpublished studies found the same prevalence of congenital malformation as the average of the published studies. We re-calculated the pooled odds ratio under these extreme assumptions.

All subgroup analyses, including anatomic location of the malformation, type of macrolides and pregnancy term, were planned *a priori* and were identified and published in the protocol registered in PROSPERO.

All analyses were carried out using the software HEpiMA version 2.1.3 [27], and STATA version 12 (Stata Corp, College Station, Tex).

3. Results

Out of 2841 publications retrieved initially, 17 cohort studies [14-16, 22-25, 28-37], and 4 case-control studies [9,11, 38-40], met our inclusion criteria and were included in the meta-analysis (Fig 1). The data of two case-control studies that encompassed different macrolides but were carried out in the same population were analyzed together and considered as one single study [38, 40]. No clinical trial was retrieved in our search. In these 21 studies, the comparator consisted of fetuses non-exposed to any drug and/or fetuses exposed to non-macrolide antibiotics or non-teratogens before birth. ORs of birth defects after prenatal exposure to macrolides in comparison with fetuses not exposed to any drug (comparison group 1) were obtained from 14 of the 21 included studies; and ORs in reference to prenatal exposure to non-macrolide antibiotics or non-teratogens (comparison group 2) were available in 13 studies.

All case-control studies were population-based and included an average number of 10483 cases and 12937 controls. The 21 studies were mainly concentrated in Europe and North America and were carried out between 1998 and 2017 (Online resources 2 and 3 and Fig 2).

The three different analyses yielded similar results (Tables 1-3). No significant association was observed between prenatal exposure to macrolides and fetal malformation, when the unexposed population was that of fetuses not exposed to any drug [$OR_{group\ 1}$: 1.05 (95%CI: 0.99, 1.12)] (Table 1). Compared to fetuses exposed to non-macrolides/non-teratogens the pooled $OR_{group\ 2}$ was 1.06 (95%CI: 1.00, 1.12)] (Tables 2). When the unexposed population was that composed of fetuses non-exposed to any drug and/or to those exposed to non-macrolide antibiotics or non-teratogens, the pooled $OR_{group\ 3}$ of any congenital malformation in babies born to women exposed to macrolides during pregnancy was 1.06 (95%CI: 1.01, 1.10) (Table 3).

Subgroup analysis in reference to fetuses unexposed to any drug before birth (comparison group 1) did not reveal any significant association between macrolides and birth defects. This lack of association was also observed in most of the subgroups in the other two analyses (comparison groups 2 and 3).

Pooled estimates of cohort studies were only significant when comparison was made in reference to the mixed population of unexposed fetuses (comparison group 3) [$OR_{group\ 3}$: 1.07 (95%CI: 1.02, 1.13)], while the case-control studies did not show any significant association between macrolides use during pregnancy and congenital malformations.

We did not detect any heterogeneity in the subgroup analysis that compared macrolides exposure to non-exposure to any drug during pregnancy (comparison group 1). When babies prenatally exposed to macrolides were compared to those exposed to non-macrolides/non-teratogenic drugs (comparison group 2) and to the mixed population of unexposed fetuses (comparison group 3), a substantial heterogeneity was detected in the studies assessing malformations in the urogenital system ($Ri_{group\ 2}$ = 0.84 and $Ri_{group\ 3}$ = 0.81), and nervous systems ($Ri_{group\ 2}$ = 0.70 and $Ri_{group\ 3}$ = 0.71), as well as in those evaluating the effect of erythromycin ($Ri_{group\ 2}$ = 0.84 and $Ri_{group\ 3}$ = 0.79). Finally, the studies involving cardiovascular malformations, digestive system malformations and exposure during the third trimester of pregnancy also harbored a substantial amount of heterogeneity when macrolides were compared to the mixed non-exposure category ($Ri_{group\ 3}$ = 0.71, $Ri_{group\ 3}$=0.44 and $Ri_{group\ 3}$=0.62, respectively).

The quality score of the 21 studies ranged between 1 and 7 points, with a median of 4. In the studies that used fetuses unexposed to any medicine as a comparator, those scoring 4 points or more showed a statistically non-significant pooled OR, similar to that of the studies with lower score [$OR_{group\ 1}$: 1.04 (95%CI: 0.95, 1.14) versus $OR_{group\ 1}$: 1.06 (95%CI: 0.98, 1.16)].

However, the pooled OR of studies scoring at least 4 points was statistically significant when macrolides were compared to fetuses exposed to other antibiotics [OR $_{group\ 2}$: 1.08 (95%CI: 1.01, 1.15)], or to the mixed population of non-exposed fetuses [OR $_{group\ 3}$: 1.07 (95%CI: 1.01, 1.13)]. This statistical significance was not observed in studies scoring less than 4 points [OR $_{group\ 2}$: 1.02 (95%CI: 0.92, 1.13) and OR $_{group\ 3}$: 1.04 (95%CI: 0.97, 1.12)].

In a similar fashion, no substantial difference was found between studies with full adjustment and those with incomplete adjustment in the three patterns of analyses.

The magnitude of the association of macrolides prenatal exposure and congenital malformations slightly varied between geographical regions in the analyses that compared fetuses exposed to macrolides to those exposed to non-macrolides (comparison group 2) and to the mixed unexposed population (comparison group 3). The association is higher in studies carried out in a European population [OR $_{group\ 2}$: 1.15 (95%CI: 1.01, 1.31) and OR $_{group\ 3}$: 1.12 (1.03, 1.22)] than in North American studies [OR $_{group\ 2}$: 1.04 (95%CI: 0.98, 1.11) and OR $_{group\ 3}$:1.03 (95%CI: 0.98, 1.09)].

In the subgroups of exposure period, macrolides were exclusively associated with a minimal increase in the odds of congenital malformations [OR $_{group\ 3}$: 1.06 (95%CI: 1.01, 1.12)] when taken during the first trimester of pregnancy. This significant association was also limited to the analysis that involved the mixed unexposed population (comparison group 3). Exposure during the third trimester did not show any significant increase in the odds when macrolides were compared to any of the three types of unexposed population.

The studies included in this meta-analysis encompass several classes of macrolides, mainly: erythromycin, azithromycin, clarithromycin and roxithromycin. Analyzing the individual effect of each type of macrolides revealed that roxithromycin presents a substantial but statistically non-significant association with fetal malformation when compared to fetuses

that were not exposed to any drug [OR $_{group\ 1}$: 2.03 (95%CI: 0.75, 5.48)], to fetuses exposed to non-macrolides or non-teratogenic antibiotics [OR $_{group\ 2}$: 1.50 (95%CI: 0.81, 2.77)], and to fetuses of both groups [OR $_{group\ 3}$: 1.63 (95%CI: 0.96, 2.75)]. The other macrolides were not found to be associated with fetal malformations when compared to any of the three unexposed groups.

Stratifying the analysis by anatomical location showed that there is no significant association in relation to prenatal exposure to macrolides and any of the body system malformations when macrolides were compared with fetuses non-exposed to any drug (comparison group 1). Increased odds of the musculoskeletal system malformation were observed when comparison was made with fetuses exposed to non-teratogenic antibiotics [OR $_{group\ 2}$: 1.21 (95%CI: 1.08, 1.35)] as well as when compared with the mixed population of unexposed fetuses [OR $_{group\ 3}$: 1.15 (95%CI: 1.05, 1.26)]. Digestive system malformations were also associated with a borderline increase in the odds of malformations when macrolides were compared with the mixed unexposed population [OR $_{group\ 3}$:1.14 (95%CI: 1.02, 1.26)]. Cardiovascular congenital malformations were not associated with prenatal exposure to macrolides when compared with fetuses unexposed to any drug and to the mixed comparison group. However, surprisingly, prenatal exposure to macrolides was associated with decreased odds of cardiovascular birth defects when compared to other antibiotics [OR $_{group\ 2}$: 0.87 (95%CI: 0.81, 0.95)].

The visual examination of the funnel plot revealed some degree of asymmetry with a larger number of studies that favor the existence of effect (right hand side of the figure) (Fig 3). However, this asymmetry was not confirmed by the Egger's regression test (p-value = 0.074) but the trim-and-fill procedure imputed 6 studies and suggested a corrected OR of 1.02 (95%CI: 1.00, 1.04).

Under the assumptions of our sensitivity analysis, there was no increase in the odds of malformations [OR: 1.00 (95%CI: 0.98, 1.03)].

4. Discussion

The etiology of birth defects is not well determined and could be related to various conditions such as prenatal exposure to certain drugs. Therefore, to determine whether macrolides´ prenatal exposure exerts an effect on the risk of congenital abnormalities and to study whether this effect varies according to the type of macrolide and to the exposure pregnancy term, we carried out a meta-analysis.

To address any potential bias due to treatment, we carried out three analyses using comparison groups that differ by their exposure to medicines. Globally, our results suggest the existence of a weak association of macrolides' prenatal use and congenital malformations, limited to exposure in early pregnancy, and musculoskeletal and digestive systems. This association was observed only in the analysis that involved mixed exposures in the comparison group

Our results are in line with a previous report which only found increased odds of digestive system birth defects based on two studies [17]. It was also suggested that erythromycin acts as motilin agonist which provokes strong gastric contractions which in turn leads to defects in the digestive system such as hypertrophy in the last trimester of pregnancy [41]. Animal studies revealed that, during the early development of the embryo, the heart is extremely susceptible to drugs that block the cardiac potassium channel (such as macrolides), which leads to bradycardia, arrhythmia and cardiac arrest, even at concentrations not affecting the maternal heart [42]. In this meta-analysis, we also reported that the musculoskeletal system might be potentially affected with macrolides prenatal exposure, but to date, there is lack of information that would justify this association. Moreover, we found that macrolides are

associated with lower odds of cardiovascular malformations, when compared to exposure to other antibiotics. This suggests, surprisingly, that macrolides could be safer than other antibiotics when used during pregnancy. A recent meta-analysis that assessed the cardiovascular safety of macrolides in reference to penicillin did not find any association between macrolides and the risk of arrhythmia or cardiovascular mortality [43].

Specific population-related effect was observed in our meta-analysis, as macrolides prenatal exposure seems to increase the odds of birth defects in the European population more than in North America. Canfield et al. showed that many congenital anomalies vary across ethnic groups [44]. This difference between the two continents could also be due to variations in the types of infection treated in these countries. In the US, macrolides are among the first-line-agents for treating respiratory infections and its prescription is approved in case of infections caused by atypical pathogens, while in Europe, atypical infections, are considered less clinically relevant, and patients with these infections would probably receive a β-lactam instead of macrolides, especially after the threats of macrolides resistance [45].

Publication bias may have plausibly affected the results of our meta-analysis, causing an overestimation of the effect. Indeed, we detected some asymmetry in the funnel plot and the trim-and-fill procedure imputed not fewer than 6 potentially unpublished studies, which yielded a corrected Odds Ratio that favored the absence of effect.

Furthermore, an overestimation of the statistical significance of our results cannot be ruled out due to the fact that multiple comparisons were carried out.

As in any meta-analysis of observational studies the results should be carefully assessed as to whether they might be biased by residual confounders which were not eliminated by adjustment. It is remarkable that only 3 out of the 21 studies adjusted for both maternal age and urinary tract infections, factors that were associated with an increased risk of non-

chromosomal congenital abnormalities in previous studies and could represent potential confounders [9, 46]. However, it is worth mentioning that the results after restriction of the analysis to the fully adjusted studies did not differ from that of the incompletely adjusted studies.

Misclassification of the outcome is highly improbable to occur for such a diagnosis. Misclassification of exposure to macrolides is also unlikely as it was well documented in more than half (13 out of 21) of the included studies in which the corresponding data were collected through medical records. In addition, although it is unlikely that recall bias may have affected our results, we recalculated the pooled OR by excluding the studies that assessed exposure using a questionnaire or an interview (and not medical records), and the results remained unaltered. However, exposure to macrolides might be misclassified if some women did not actually take the macrolides that were prescribed.

Due to the absence of data in the original studies, the results of our meta-analysis are limited by the absence of dose-response analysis. In addition, due to unavailability of data, we did not consider malformation outcomes in abortions or stillbirths. Therefore, we cannot rule out a mutagenic effect of macrolides that could have caused abortions or stillbirths.

5. Conclusion

In summary, our meta-analysis showed that the increase in the odds of birth defects among women who consumed macrolides during their pregnancy is very low. However, a harmful effect of macrolides cannot be ruled out, especially for the musculoskeletal and digestive systems. Risk-benefit research is needed to address the question of whether macrolide prescription should be restricted during pregnancy.

Authors´ contributions

Conception and design of the study: Bahi Takkouche and Mahyar Etminan. Conceptualization of the manuscript and review and synthesis of the literature: Narmeen Mallah. Data extraction: Narmeen Mallah and Hamid Reza Tohidinik. Coordination and supervision of data extraction and analysis: Bahi Takkouche and Adolfo Figueiras. All authors made substantial contribution to the interpretation of data, critically reviewed the manuscript and approved its submission for publication.

Compliance with Ethical Standards:

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Data Sharing: All data generated or analyzed during this study are included in this published article and its supplementary information file (Online Resource 1).

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Figures

Fig. 1 Flow chart of screening for literature about macrolides' prenatal exposure and congenital malformation

Fig. 2 Study specific and pooled odds ratios of prenatal exposure to macrolides and congenital malformations

Fig. 3 Funnel plot of prenatal macrolides' exposure and any congenital malformation

Supplemental Information

Online Resource 1 (Table ESM_1). Dataset of extracted and calculated OR of general and specific congenital malformations classified by the comparator group, continent, OR adjustment status, quality assessment score, body systems, exposure period and type of macrolide.

Online Resource 2 (Table ESM_2): represents the characteristics of cohort studies of macrolides´ intake and congenital malformations

Online Resource 3 (Table ESM_3): represents the characteristics of case-control studies of macrolides´ intake and congenital malformations

Table 1 Pooled Odds Ratios (ORs) and 95% Confidence Intervals (CIs) of macrolides' intake and congenital malformations (comparison group 1: fetuses not exposed to any drug)

	Number of studies	OR (95% CI) Fixed effects	OR (95% CI) Random effects	Ri*	Q test (p value)
Any congenital malformation					
All studies	14	1.05 (0.99, 1.12)	1.05 (0.99, 1.12)	0.00	0.81
Study Design					
Cohort	11	1.07 (0.99, 1.15)	1.07 (0.99, 1.15)	0.00	0.90
Case-control studies	3	1.02 (0.92, 1.14)	1.01 (0.88, 1.17)	0.39	0.21
Anatomic location					
Cardiovascular	8	1.03 (0.93, 1.14)	1.05 (0.90, 1.22)	0.48	0.07
Head and Neck	4	1.27 (0.94, 1.72)	1.27 (0.94, 1.72)	0.00	1.00
Musculoskeletal system	5	1.06 (0.91, 1.24)	1.06 (0.85, 1.31)	0.33	0.27
Digestive system	8	1.13 (0.97, 1.31)	1.12 (0.9, 1.33)	0.18	0.30
Urogenital system	5	1.00 (0.80, 1.24)	0.98 (0.71, 1.35)	0.43	0.17
Nervous system	4	1.14 (0.86, 1.52)	1.12 (0.81, 1.55)	0.19	0.28
Adjustment					
Full	2	1.04 (0.94, 1.15)	1.04 (0.94, 1.15)	0.00	0.50
Incomplete	12	1.06 (0.98, 1.15)	1.06 (0.98, 1.15)	0.00	0.75
Quality score					
≥ 4	7	1.04 (0.95, 1.14)	1.04 (0.95, 1.14)	0.00	0.78
< 4	7	1.06 (0.98, 1.16)	1.06 (0.98, 1.16)	0.00	0.56
Macrolide exposure period					
First trimester	11	1.07 (1.00, 1.14)	1.07 (1.00, 1.14)	0.00	0.93
Third trimester	3	1.00 (0.79, 1.28)	1.08 (0.73, 1.59)	0.60	0.11
Geographic location					
Europe	6	1.10 (0.98, 1.23)	1.10 (0.98, 1.23)	0.00	0.71
North America	6	1.03 (0.96, 1.12)	1.03 (0.96, 1.12)	0.00	0.45
Type of treatment					
Erythromycin	7	1.05 (0.97, 1.15)	1.05 (0.97, 1.15)	0.00	0.46
Azithromycin	2	1.15 (0.97, 1.36)	1.15 (0.97, 1.36)	0.00	0.37
Clarithromycin	2	1.10 (0.88, 1.38)	1.10 (0.88, 1.38)	0.00	0.84
Roxithromycin	2	2.03 (0.75, 5.48)	2.03 (0.75, 5.48)	0.00	0.79

*Ri: Proportion of total variance due to between-study variance.

Table 2 Pooled Odds Ratios (ORs) and 95% Confidence Intervals (CIs) of macrolides' intake and congenital malformations. (comparison group 2: fetuses exposed to non-teratogenic drugs/non-macrolides)

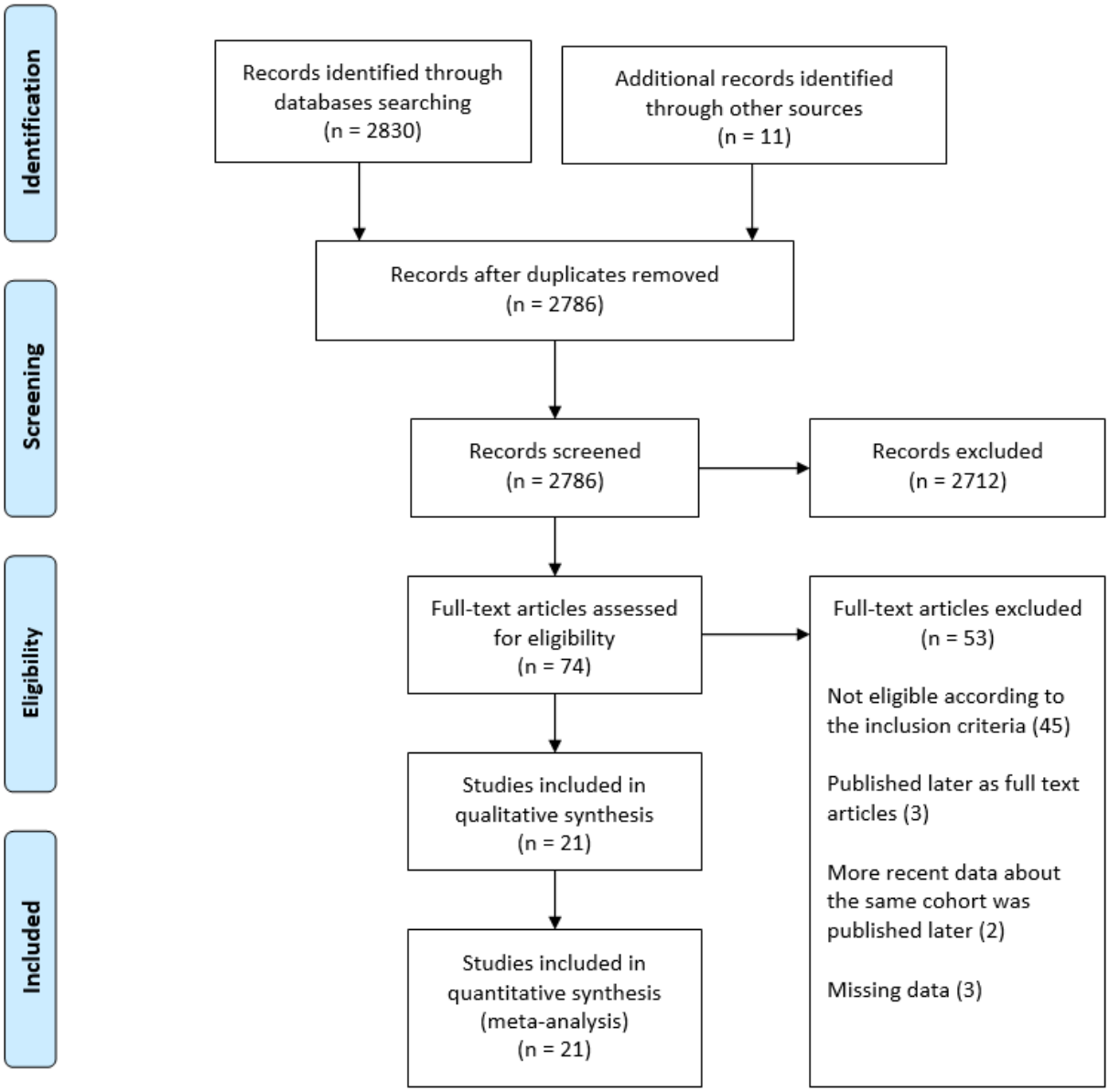
	Number of studies	OR (95% CI) Fixed effects	OR (95% CI) Random effects	Ri*	Q test (p value)
Any congenital malformation					
All studies	13	1.06 (1.00, 1.12)	1.06 (1.00, 1.12)	0.00	0.88
Study Design					
Cohort	11	1.07 (1.00, 1.15)	1.07 (1.00, 1.15)	0.00	0.86
Case-control studies	2	1.03 (0.93, 1.14)	1.03 (0.93, 1.14)	0.00	0.36
Anatomic location					
Cardiovascular	7	0.87 (0.81, 0.95)	0.93 (0.80, 1.07)	0.50	0.10
Head and Neck	4	1.08 (0.90, 1.29)	1.05 (0.78, 1.42)	0.54	0.12
Musculoskeletal system	4	1.21 (1.08, 1.35)	1.21 (1.08, 1.35)	0.00	0.85
Digestive system	5	1.14 (0.98, 1.33)	1.11 (0.90, 1.38)	0.44	0.15
Urogenital system	4	0.89 (0.76, 1.05)	0.92 (0.60, 1.39)	0.84	0.001
Nervous system	4	1.14 (0.92, 1.42)	1.05 (0.68, 1.62)	0.70	0.03
Adjustment					
Full	2	1.05 (0.96, 1.15)	1.05 (0.96, 1.15)	0.00	0.48
Incomplete	11	1.07 (0.99, 1.15)	1.07 (0.99, 1.15)	0.00	0.84
Quality score					
≥ 4	8	1.08 (1.01, 1.15)	1.08 (1.01, 1.15)	0.00	0.74
< 4	5	1.02 (0.92, 1.13)	1.02 (0.92, 1.13)	0.00	0.86
Macrolide exposure period					
First trimester	9	1.05 (0.99, 1.12)	1.05 (0.99, 1.12)	0.00	0.83
Third trimester	2	1.33 (0.99, 1.79)	1.33 (0.99, 1.79)	0.00	0.34
Geographic location					
Europe	7	1.15 (1.01, 1.31)	1.15 (1.01, 1.31)	0.00	0.90
North America	6	1.04 (0.98, 1.11)	1.04 (0.98, 1.11)	0.00	0.81
Type of treatment					
Erythromycin	6	0.92 (0.86, 0.99)	0.99 (0.83, 1.18)	0.84	0.00
Azithromycin	6	1.08 (0.98, 1.19)	1.08 (0.98, 1.19)	0.00	0.63
Clarithromycin	4	0.92 (0.82, 1.04)	0.92 (0.82, 1.04)	0.00	0.95
Roxithromycin	3	1.50 (0.81, 2.77)	1.74 (0.69, 4.37)	0.53	0.13

*Ri: Proportion of total variance due to between-study variance.

Table 3 Pooled Odds Ratios (ORs) and 95% Confidence Intervals (CIs) of macrolides' intake and congenital malformations. (Comparison group 3: mixed population of unexposed fetuses)

	Number of studies	OR (95% CI) Fixed effects	OR (95% CI) Random effects	Ri*	Q test (p value)
Any congenital malformation					
All studies	21	1.06 (1.01, 1.10)	1.06 (1.01, 1.10)	0.00	0.86
Study Design					
Cohort	17	1.07 (1.02, 1.13)	1.07 (1.02, 1.13)	0.00	0.93
Case-control studies	4	1.03 (0.95, 1.11)	1.02 (0.94, 1.12)	0.14	0.35
Anatomic location					
Cardiovascular	11	0.93 (0.87, 0.98)	1.03 (0.89, 1.18)	0.71	0.002
Head and Neck	5	1.13 (0.96, 1.32)	1.12 (0.93, 1.35)	0.19	0.31
Musculoskeletal system	6	1.15 (1.05, 1.26)	1.15 (1.05, 1.26)	0.00	0.40
Digestive system	9	1.14 (1.02, 1.26)	1.10 (0.94, 1.28)	0.44	0.08
Urogenital system	6	0.93 (0.81, 1.05)	0.96 (0.69, 1.34)	0.81	0.001
Nervous system	5	1.15 (0.97, 1.36)	1.02 (0.72, 1.47)	0.71	0.01
Adjustment					
Full	3	1.05 (0.98, 1.12)	1.04 (0.97, 1.12)	0.00	0.50
Incomplete	18	1.06 (1.00, 1.12)	1.06 (1.00, 1.12)	0.00	0.84
Quality score					
≥ 4	12	1.07 (1.01, 1.13)	1.07 (1.01, 1.13)	0.00	0.78
< 4	9	1.04 (0.97, 1.12)	1.04 (0.97, 1.12)	0.00	0.70
Macrolide exposure period					
First trimester	16	1.06 (1.01, 1.11)	1.06 (1.01, 1.11)	0.00	0.95
Third trimester	4	1.12 (0.93, 1.35)	1.15 (0.85, 1.56)	0.62	0.05
Geographic location					
Europe	10	1.12 (1.03, 1.22)	1.12 (1.03, 1.22)	0.00	0.86
North America	9	1.03 (0.98, 1.09)	1.03 (0.98, 1.09)	0.00	0.74
Type of treatment					
Erythromycin	9	0.96 (0.91, 1.01)	1.00 (0.88, 1.14)	0.79	0.00
Azithromycin	6	1.09 (1.00, 1.19)	1.09 (1.00, 1.19)	0.00	0.73
Clarithromycin	5	0.96 (0.86, 1.07)	0.96 (0.86, 1.07)	0.00	0.97
Roxithromycin	5	1.63 (0.96, 2.75)	1.66 (0.95, 2.89)	0.08	0.36

*Ri: Proportion of total variance due to between-study variance.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Screening
Eligibility
Included
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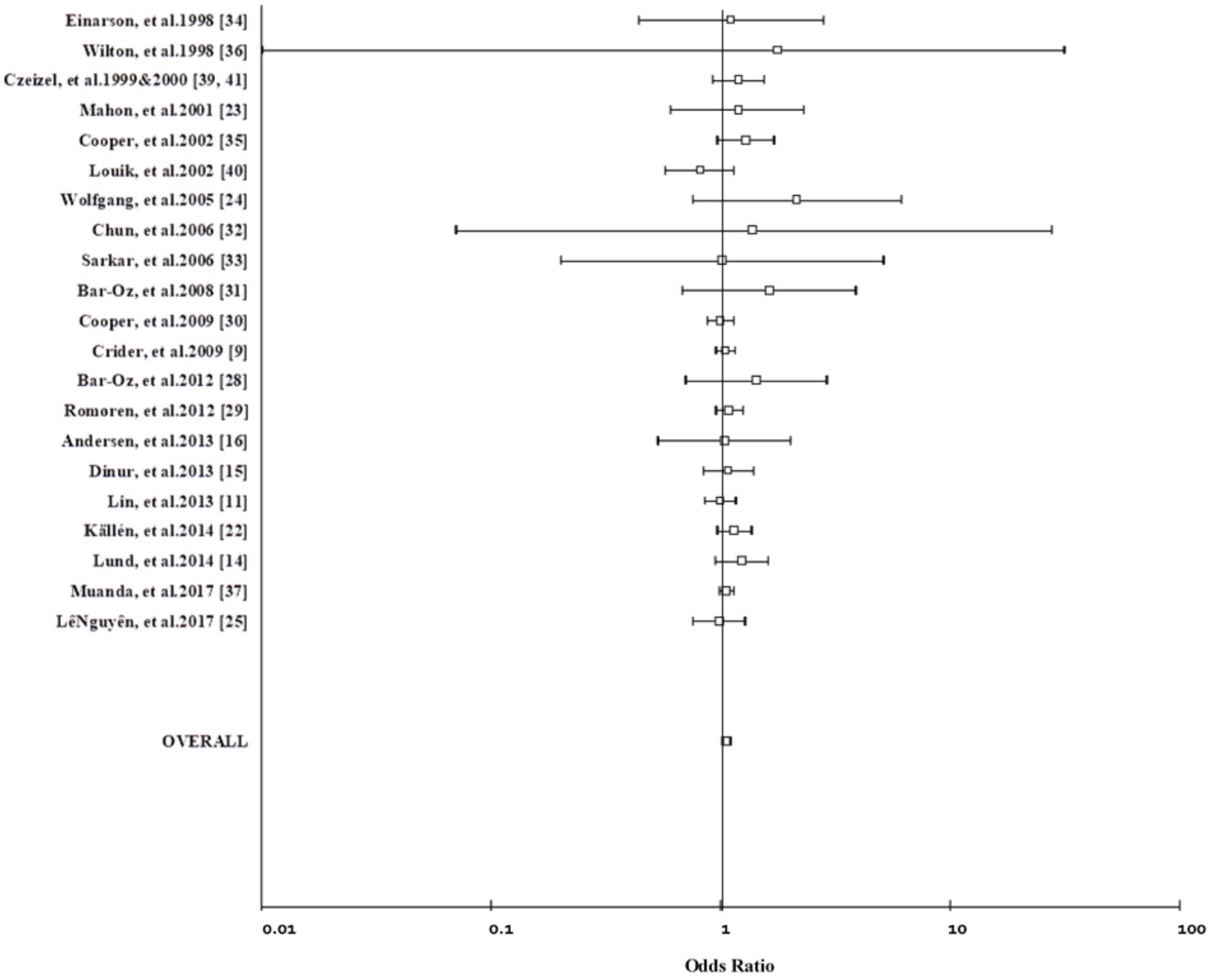
Additional records identified through other sources (n = 11)
Records after duplicates removed (n = 2786)
Records screened (n = 2786)
Records excluded (n = 2712)
Full-text articles assessed for eligibility (n = 74)
Full-text articles excluded (n = 53)
Not eligible according to the inclusion criteria (45)
Published later as full text articles (3)
More recent data about the same cohort was published later (2)
Missing data (3)
Studies included in qualitative synthesis (n = 21)
Studies included in quantitative synthesis (meta-analysis) (n = 21)

Figure 1.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Wilton, et al.1998 [36]
Czeizel, et al.1999&2000 [39, 41]
Mahon, et al.2001 [23]
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Louik, et al.2002 [40]
Wolfgang, et al.2005 [24]
Chun, et al.2006 [32]
Sarkar, et al.2006 [33]
Bar-Oz, et al.2008 [31]
Cooper, et al.2009 [30]
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Lund, et al.2014 [14]
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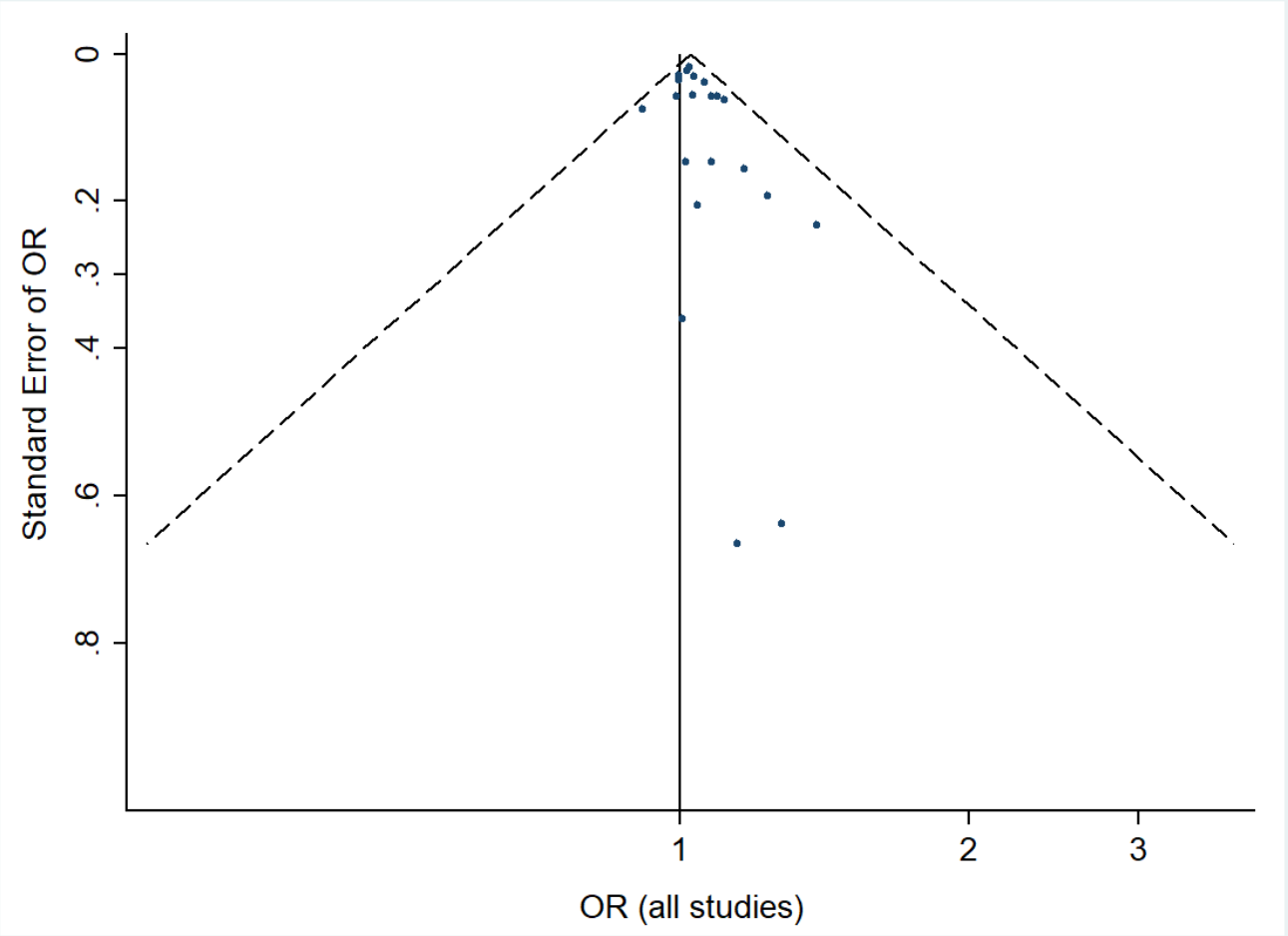
0.1
1
10
100
Odds Ratio

Figure 2.



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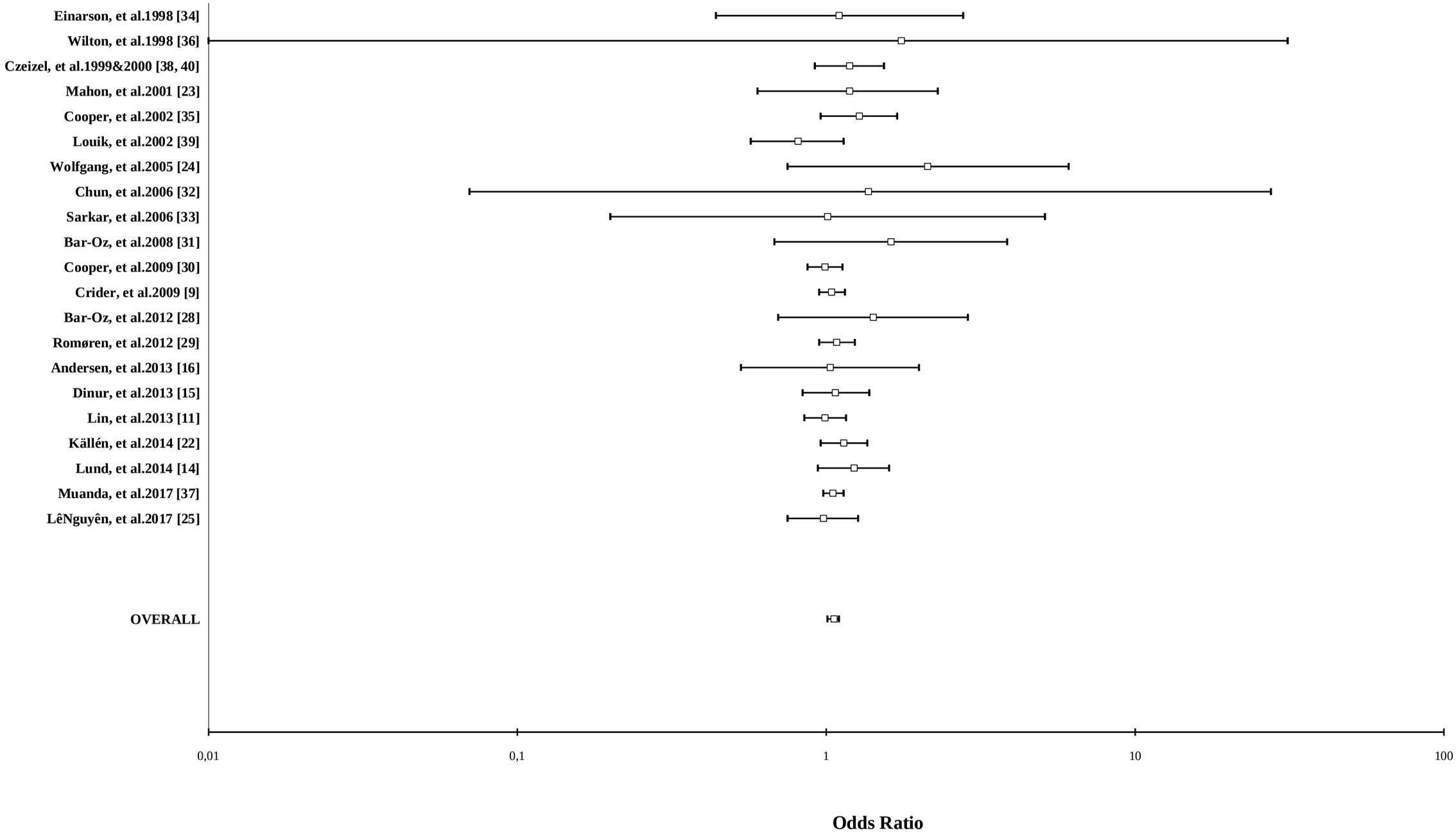
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Lin, et al.2013 [11]
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LêNguyên, et al.2017 [25]
OVERALL
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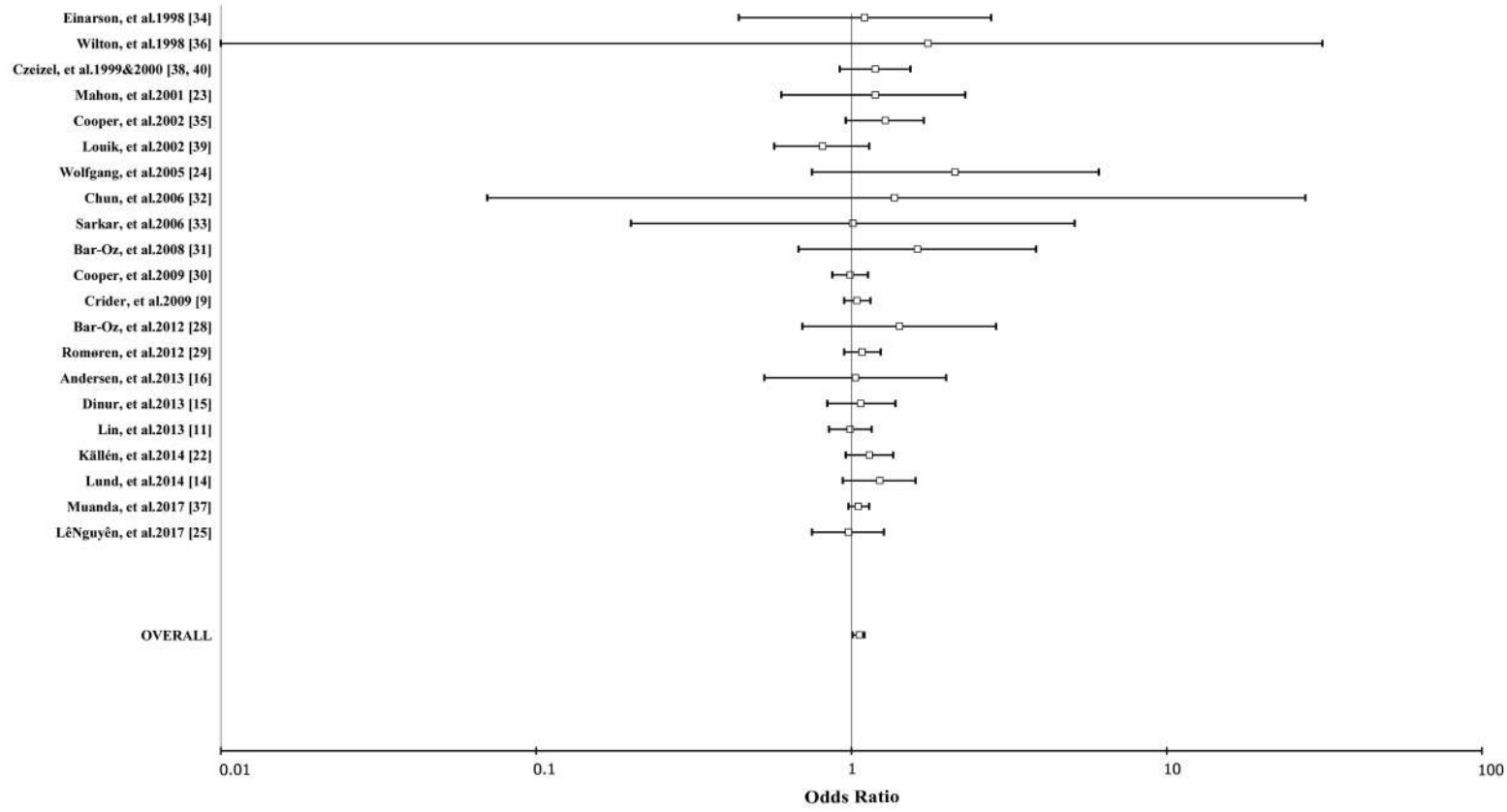
Odds Ratio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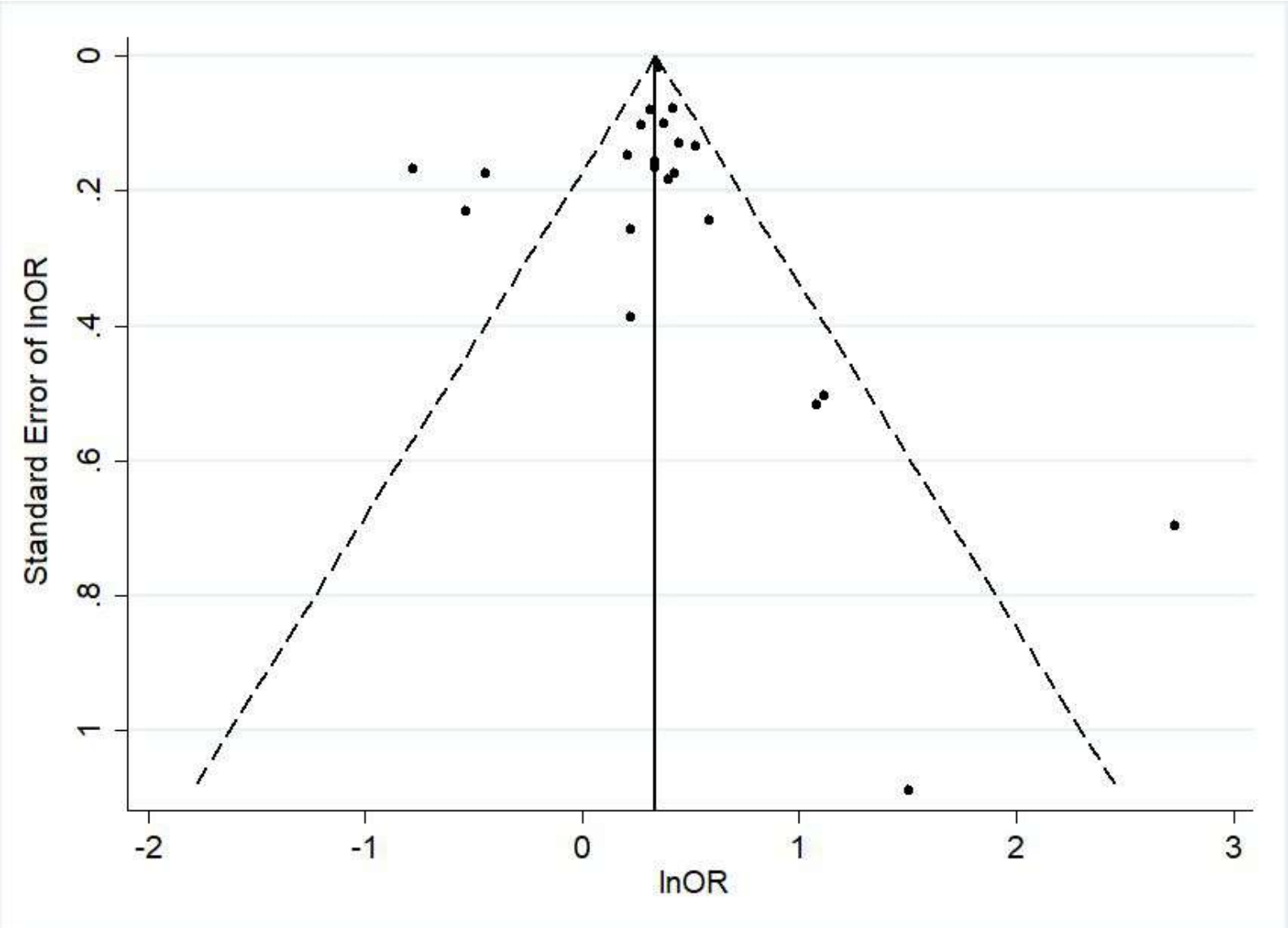
Lin, et al.2013 [11]
Källén, et al.2014 [22]
Lund, et al.2014 [14]
Muanda, et al.2017 [37]
LêNguyên, et al.2017 [25]
OVERALL
0.01
0.1
1
10
100
Odds Ratio


Standard Error of lnOR
0
.2
.4
.6
.8
1
-2
-1
0
1
2
3
lnOR

Online Resource 1. Dataset of extracted and calculated OR (data)

Source	RR_all	UpperCI_all	unexposedCtrl	RR_UnexposedCtrl	UpperCI_UnexposedCtrl	ExposedCtrl	RR_ExposedCtrl	UpperCI_ExposedCtrl	Design	EU.America	EU.noEU	Quality4pts
Muanda2017	1.05	1.14	yes	1.08	1.23	yes	1.04	1.14	cohort	North America	NON EUROPEAN	>or=4
LêNguyên2017	0.98	1.27	yes	1.02	1.46	yes	0.93	1.37	cohort	Europe	EUROPE	<4
Källén2014	1.14	1.36	yes	1.14	1.36	no			cohort	Europe	EUROPE	<4
Lund2014	1.23	1.6	yes	1.23	1.76	yes	1.23	1.83	cohort	Europe	EUROPE	<4
Andersen2013	1.03	2.00	yes	1.03	2.00	no			cohort	Europe	EUROPE	>or=4
BahatDinur2013	1.07	1.38	yes	1.07	1.38	no			cohort		NON EUROPEAN	>or=4
Bar-Oz2012	1.42	2.88	no			yes	1.42	2.88	cohort	Europe	EUROPE	>or=4
Romøren2012	1.08	1.24	yes	1.02	1.23	yes	1.15	1.38	cohort	Europe	EUROPE	>or=4
Cooper2009	0.99	1.13	yes	0.92	1.16	yes	1.03	1.21	cohort	North America	NON EUROPEAN	>or=4
Bar-Oz2008	1.62	3.86	no			yes	1.62	3.86	cohort	Europe	EUROPE	>or=4
Chun2006	1.37	27.57	yes	1.37	27.57	no			cohort		NON EUROPEAN	>or=4
Sarkar2006	1.01	5.11	no			yes	1.01	5.11	cohort	North America	NON EUROPEAN	>or=4
Wolfgang2005	2.13	6.1	yes	2.13	6.1	no			cohort	Europe	EUROPE	>or=4
Cooper2002	1.28	1.7	no			yes	1.28	1.7	cohort	North America	NON EUROPEAN	>or=4
Mahon2001	1.19	2.3	yes	1.19	2.3	no			cohort	North America	NON EUROPEAN	<4
Einarson1998	1.1	2.78	no			yes	1.1	2.78	cohort	North America	NON EUROPEAN	<4
Wilton1998	1.75	31.23	no			yes	1.75	31.23	cohort	Europe	EUROPE	<4
Lin2013	0.99	1.16	yes	0.99	1.16	no			case-control	North America	NON EUROPEAN	<4
Crider2009	1.04	1.15	yes	1.12	1.32	yes	1.01	1.13	case-control	North America	NON EUROPEAN	<4
Louik2002	0.81	1.14	yes	0.81	1.14	no			case-control	North America	NON EUROPEAN	<4
Czeizel1999&2000	1.19	1.54	no			yes	1.16	1.53	case-control	Europe	EUROPE	>or=4

Online Resource 2. Characteristics of cohort studies of macrolides´ intake and congenital malformations

Source	Type of Macrolide	Country	Type of malformation	Exposure Period (months of pregnancy)	Comparison Type and OR (95% CI)	Cases/ Cohort Size	General OR (95% CI)	Adjustment, Matching, and Restriction Factors
Muanda FT, et al. 2017 [1]	azithromycin, erythromycin, clarithromycin	Quebec	Cardiac, digestive, head and neck, musculoskeletal, nervous, respiratory, urogenital	1 – 3	Unexposed to any antibiotic: 1.08 (0.95, 1.23) Exposed to non-macrolide antibiotics: 1.04 (0.95, 1.14)	13, 852/139,938 1627/15469	1.05 (0.98, 1.14)	Maternal age, urinary tract infections, socio-demographic variables, chronic maternal illness, endometriosis and other maternal infections, healthcare utilization, year of delivery, infant's gender
Lê Nguyên T, et al. 2017 [2]	macrolides	France	Congenital malformations	1 – 3	Unexposed to any antibiotic: 1.02 (0.71, 1.46) Treated with penicillin: 0.93 (0.63, 1.37)	47/62,846 47/12,193	0.98 (0.75, 1.27)	Maternal age, long-term maternal illness, gestity, parity, multiple pregnancy
Källén B, et al. 2014 [3]	erythromycin	Sweden	Any, cardiac	1 – 3	Unexposed to any antibiotic: 1.14 (0.96, 1.36)	70339/1575847	1.14 (0.96, 1.36)	Maternal age, year of delivery, parity, smoking, obesity
Lund M, et al. 2014 [4]	azithromycin, clarithromycin, erythromycin, roxithromycin, spiramycin,	Denmark	Infantile hypertrophic pyloric stenosis	• 1 – 6 • 6 – 9	Unexposed to any antibiotic: 1.23 (0.85, 1.76) Exposed to non-macrolide antibiotics: 1.23 (0.83, 1.83)	30/315569 159/60732	1.23 (0.94, 1.60)	Birth order, infant's sex, calendar period, current age of the infant
Andersen JT, et al. 2013 [5]	clarithromycin	Denmark	Cardiac, musculoskeletal, urogenital	1 – 3	Unexposed to any antibiotic: 1.03 (0.53, 2.00)	24817/705837	1.03 (0.53, 2.00)	Maternal age, education, number of previous births, economical status

Online Resource 2. Characteristics of cohort studies of macrolides´ intake and congenital malformations (continued)

Source	Type of Macrolide	Country	Type of malformation	Exposure Period (months of pregnancy)	Comparison Type and OR (95% CI)	Cases/ Cohort Size	General OR (95% CI)	Adjustment, Matching, and Restriction Factors
Dinur AB, et al. 2013 [6]	azithromycin, clarithromycin, erythromycin, roxithromycin	Israel	Cardiac, digestive	1 – 3	Unexposed to any antibiotic: 1.07 (0.84, 1.38)	---/105492	1.07 (0.84, 1.38)	Maternal age, year of delivery, parity, ethnicity, chronic maternal illness
Bar-Oz B, et al. 2012 [7]	azithromycin, roxythromycin, clarithromycin	• Czech Republic • Germany • Israel • Italy • Netherlands	Cardiac	1 – 3	Exposed to non-teratogenic agents: 1.42 (0.70, 2.88)	32/1146	1.42 (0.70, 2.88)	Maternal age, smoking, alcohol consumption, previous abortions, previous child with structural anomaly, macrolide exposure
Romøren M, et al. 2012 [8]	erythromycin, azithromycin, clarithromycin, spiramycin	Norway	Any, Cardiac	1 – 3	Unexposed to any antibiotic: 1.02 (0.86, 1.23) Exposed to non-macrolide antibiotics: 1.15 (0.96, 1.38)	8865/178142 413/9069	1.08 (0.95, 1.24)	Maternal age, urinary tract infections, chronic maternal illness, parity, marital status, smoking, pregnancy supplement, previous abortions
Cooper WO, et al. 2009 [9]	azithromycin, erythromycin	United States	Any, digestive, head and neck, nervous, musculoskeletal, urogenital	• 1 – 3 • 1 – 9	Unexposed to any antibiotic: 0.92 (0.73, 1.16) Exposed to non-macrolide antibiotics: 1.03 (0.88, 1.21)	869/30,049 589/7471	0.99 (0.87, 1.13)	Maternal age, year of delivery, race, rural residence, economical status, chronic maternal illness, filling of prescriptions of other known teratogens
Bar-Oz B, et al. 2008 [10]	azithromycin, roxythromycin, clarithromycin	• Croatia • Israel	Cardiac	1 – 3	Exposed to non-macrolide antibiotics: 1.62 (0.68, 3.86)	32/1066	1.62 (0.68, 3.86)	Unadjusted

Online Resource 2. Characteristics of cohort studies of macrolides´ intake and congenital malformations (continued)

Source	Type of Macrolide	Country	Type of malformation	Exposure Period (months of pregnancy)	Comparison Type and OR (95% CI)	Cases/ Cohort Size	General OR (95% CI)	Adjustment, Matching, and Restriction Factors
Chun JY, et al. 2006 [11]	roxythromycin	South Korea	Major	1 – 3	Unexposed to any teratogenic agent: 1.37 (0.07, 27.57)	3/187	1.37 (0.07, 27.57)	Maternal age, gravity
Sarkar M, et al. 2006 [12]	azithromycin	Canada	Major	1 – 3	Exposed to any non-teratogen: 1.01 (0.20, 5.11)	6/227	1.01 (0.20, 5.11)	Maternal age, gestational age at call, smoking, alcohol consumption
Wolfgang P, et al. 2005 [13]	roxithromycin	Hungary	Congenital anomalies	1 – 3	Unexposed to any antibiotic: 2.13 (0.75, 6.1)	15/275	2.13 (0.75, 6.1)	Maternal age, gestational age at call
Cooper WO, et al. 2002 [14]	erythromycin, non-erythromycin, lincomycin, clindamycin, clarithromycin, azithromycin, dirithromycin	United States	Digestive	• 6 – 9 • 1 – 9	Exposed to non-macrolide antibiotics: 1.28 (0.96, 1.70)	679/260,799	1.28 (0.96, 1.70)	Maternal age, education, geographic residence, use of other antibiotics, infant´s gender, infant´s race, birth order, year of delivery, infant´s postnatal prescriptions for erythromycin
Mahon BE, et al. 2001 [15]	macrolides	United States	Digestive	1 – 9	Unexposed to any antibiotic: 1.19 (0.6, 2.3)	43/14,876	1.19 (0.6, 2.3)	Birth weights, gestational age
Einarson A, et al. 1998 [16]	clarithromycin	Canada	Major	1 – 3	Exposed to non-teratogenic antibiotics: 1.1 (0.44, 2.78)	19/266	1.1 (0.44, 2.78)	Maternal age, smoking, alcohol consumption
Wilton LV, et al. 1998 [17]	azithromycin	United Kingdom	Congenital anomalies	1 – 3	Exposed to non-macrolide antibiotics: 1.75 (0.01, 31.23)	14/556	1.75 (0.01, 31.23)	Unadjusted

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Online Resource 3. Characteristics of case-control studies of macrolides´ intake and congenital malformations

Source	Type of Macrolide	Country	Type of malformation	Exposure Period (months of pregnancy)	Comparison Type and OR (95% CI)	Cases/Controls	General OR (95% CI)	Adjustment, Matching, and Restriction Factors
Lin KJ, et al. 2013 [1]	Any macrolide, erythromycin, non-erythromycin	• Canada • United States	Cardiac, digestive, head and neck, musculoskeletal, nervous, respiratory, urogenital	• 1 – 3 • 3 – 6 • 6 – 9	Unexposed to any antibiotic: 0.99 (0.85, 1.16)	4867/6,952	0.99 (0.85, 1.16)	Maternal age, calendar year when they were ascertained, race, education, geographic residence, obesity, family history of congenital malformations or diabetes mellitus, smoking, pregnancy supplement, multiple pregnancy, urinary tract infections, maternal chronic illness
Crider KS, et al. 2009 [2]	erythromycin	United States	Cardiac, digestive, head and neck, musculoskeletal, nervous, urogenital	1 – 3	Unexposed to any antibiotic: 1.12 (0.96, 1.32) Exposed to non-macrolide antibiotics: 1.01 (0.91, 1.13)	13155/4941 1384/516	1.04 (0.95, 1.15)	Maternal age, race, education, obesity, gestational age at call, pregnancy supplements, smoking, alcohol consumption
Louik C, et al. 2002 [3]	erythromycin	• Canada • United States	Digestive	• 1 – 6 • 6 – 9	Unexposed to any antibiotic: 0.81 (0.57, 1.14)	1,044/1704	0.81 (0.57, 1.14)	Maternal age, geographic region, study period, parity, infant´s gender, gestational age
Czeizel AE, et al. 2000 [4] [&] Czeizel AE, et al. 1999 [5]	erythromycin, spiramyicn, roxithromycin, oleandomycin, josamycin,	Hungary	Cardiac, head and neck, musculoskeletal, urogenital, nervous, others	• 1 – 3 • 1 – 9	Exposed to other agents (not macrolides): 1.19 (0.92, 1.54)	22,865/38,151	1.19 (0.92, 1.54)	Maternal age, urogenital disorders, birth order, maternal chronic illness, other drug uses

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