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Title:
The Effect of Maternal Use of Macrolides, Antifungals, and Antivirals/ Antiretrovirals on Non-Syndromic Orofacial Clefts

A Systematic Review and Meta-analysis

The Effect of Maternal Use of Macrolides, Antifungals, and Antivirals/ Antiretrovirals on Non-Syndromic Orofacial Clefts: A Systematic Review and Meta-analysis

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A dissertation submitted to the University of Bristol in accordance with the of the degree of Doctor of Dental Surgery by advanced study in Orthodontics in the Faculty of Health Sciences.

School of Oral and Dental Sciences

July 2020

Word count (30163)

ABSTRACT

Objective:

To assess the risk of non-syndromic orofacial cleft after exposure to macrolides, antifungals, and antivirals/ antiretrovirals during pregnancy.

Search Strategy:

MEDLINE, EMBASE, and the Cochrane library were searched up to April 30, 2019.

Search Criteria:

Relevant English language studies investigating foetal adverse outcomes after *in utero* exposure to macrolides, antifungals, and antivirals/ antiretrovirals.

Data Collection and Analysis:

Studies were screened by two reviewers. Data was extracted, study quality was assessed, and pooled estimates were calculated. A random effects meta-analysis was conducted to estimate the effect of macrolides and antifungals on the risk of developing a cleft. Antivirals/ antiretrovirals were not included in the meta-analysis as it was not possible to extract the data from the papers identified.

Main Results:

Overall, nine case-control studies and eight cohort studies met the inclusion criteria. Eight of the seventeen studies were included in the meta-analysis having excluded those pertaining to antivirals/ antiretrovirals and limiting to those measures that were either an "Odds Ratio" or "Prevalence Odds Ratio". The data indicated that erythromycin and fluconazole exposure during pregnancy is not associated with an increased risk of cleft lip and palate. For antivirals and antiretrovirals, no conclusion could be reached due to the high level of heterogeneity within the studies.

Conclusions:

Exposure to macrolides and antifungals was not associated with an increased risk of oral cleft. Further studies are required to investigate whether antivirals and antiretrovirals pose a risk during pregnancy.

DEDICATION AND ACKNOWLEGMENTS

To Allah for giving me the strength and wisdom to surpass all obstacles I have encountered throughout these years. To my beloved country, Kuwait, for giving me the chance to fulfil my dream in becoming an orthodontist. To my backbone, my family and friends, for their moral support and belief in that I can succeed in whatever I put my mind to. And to my daughter, Fatemah, who has given me the strength to keep going. Without them, I would not be where I am today.

To Professor Anthony Ireland and Professor Jonathan Sandy, whom I would like to extend my sincere gratitude for offering their time and effort. Their continued support and encouragement, as well as, sharing their vast knowledge has helped me in writing this dissertation. To Professor Martyn Sherriff, for his tremendous help in offering his statistical expertise.

To Nadine Homoud, a close friend and colleague and the second reviewer of this paper. I could not have gone through all of this without her by my side. And finally, to all the Bristol DDS Orthodontic Postgraduate Students who have made this challenging journey a worthwhile one.

AUTHORS DECLARATION

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Taught Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, this work is my own work. Work done in collaboration with, or with the assistance of others, is indicated as such. I have identified all material in this dissertation which is not my own work through appropriate referencing and acknowledgment. Where I have quoted or otherwise incorporated material, which is the work of others, I have included the source in the references. Any view expressed in the dissertation other than reference material, are those of the author.

Zainab Mustafa Alsaffar

July 17, 2020

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LIST OF ABBREVIATIONS

CLP	Cleft lip and palate
OFC	Orofacial cleft
NSOFC	Non-syndromic orofacial cleft
CAs	Congenital abnormalities
CL	Cleft lip
CP	Cleft palate
CL/P	Cleft lip with or without cleft palate
OC	Oral cleft
WHO	World Health Organisation
ECM	Extracellular matrix
GF	Growth factor
FGF	Fibroblast growth factor
TGF	Transforming growth factor
TGFβ-3	Transforming growth factor beta-3
FAS	Foetal alcohol syndrome
FRS	Foetal retinoid syndrome
MCMs	Major congenital malformations
PTU	Propylthiouracil
MMI	Methiamazole
CMZ	Carbimazole
NAEEP	National Education and Prevention Program
BMI	Body mass index

DM	Diabetes mellites
NICE	National Institute of Health and Care Excellence
TGA	Transposition of the Great Arteries
AVSD	Atrioventricular Septal Defect
TOF	Tetralogy of Fallot
HLHS	Hypoplastic Left Heart Syndrome
NHS	National Health Service
FASP	Foetal anomaly screening programme
AED	Anti-epileptic drugs
TMP	Topiramate
CASP	Critical Appraisal Skills Programme
OR	Odds ratio
AOR	Adjusted odds ratio
POR	Prevalence odds ratio
CI	Confidence interval
FDA	Food and Drug Administration
CDC	Centres for Disease Control and Prevention
HIV	Human immunodeficiency viruses
CHB	Chronic hepatitis B
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses

1.0 INTRODUCTION

Cleft lip and palate (CLP) is a common congenital malformation in humans, which arises during facial development. The aetiology is unknown, but like other disorders, it is likely to have both genetic and environmental influences (Mossey *et al.*, 2009; Dixon *et al.*, 2011). Orofacial clefts (OFC) can present as either an isolated trait or as part of a syndromic condition. Treatment of these congenital malformations is crucial and requires comprehensive medical and behavioural interventions, causing both a psychosocial and economic burden among the affected individual and their families (Wehby and Cassell, 2010).

During embryonic development, the maternal environment is crucial and is influenced by nutrition, medications, and lifestyle. In the first trimester of pregnancy the craniofacial complex develops, and either deficits or excesses of nutritional intake during the first trimester of pregnancy can influence the formation of CLP. Therefore, for pregnant women or women trying to conceive, a diet high in folate or folic acid supplements is recommended with avoidance of excessive intake of vitamin A (Loffredo *et al.*, 2001; Rothman *et al.*, 1995).

The adverse effects of medicinal intake on CLP are not as clear cut. The literature contains a mix of methodologies and investigations in determining what influence medications might have in the formation of cleft. This systematic review and meta-analysis aimed to investigate whether *in utero* foetal exposure to macrolides, antifungals, or antivirals/ antiretrovirals is associated with an increased risk of non-syndromic orofacial clefts (NSOFC). It also aimed to assess whether time of exposure plays a role in the development of cleft.

2.0 REVIEW OF THE LITERATURE

2.1 Cleft Lip and Palate

A cleft is a gap in the upper lip, the palate, or a combination of these structures. Orofacial clefts are categorised as syndromic if they are associated with other congenital abnormalities (CAs), or NSOFC if they occur in isolation with no other structural or developmental abnormalities. Some of the syndromes that have been associated with CLP include: Van der Woude, Pierre Robin sequence, Treacher Collins, Stickler, and 22q11 deletion syndrome (Cohen, 1978). Orofacial clefts develop due to a failure of closure of specific facial structures during weeks 5-9 of gestation (vide infra 2.3 and 2.4).

Moreover, clefts vary in severity and present as different phenotypes: isolated cleft lip (CL), isolated cleft palate (CP), and CLP. Approximately 70% of cleft lip with or without cleft palate (CL/P) cases and 50% of CP cases are non-syndromic (Mossey *et al.*, 2009). Clefts can be further classified according to the extent of tissue involvement: unilateral, bilateral, incomplete, and complete (Mossey *et al*., *2009*). As illustrated in Figure 1, CL/P affecting one side of the face is said to be unilateral, whereas bilateral affects both sides. Complete cleft refers to a cleft affecting the entire anatomical structure, such as the lip, alveolus, and/or palate. Incomplete cleft, on the other hand, involves only part of these structures. Incomplete cleft may present as a notch in the lip and/or alveolus, or a cleft involving only a portion of the palate. A minor form of CL, known as microform CL, may also develop. This phenotype presents as a vertical groove of the upper lip.

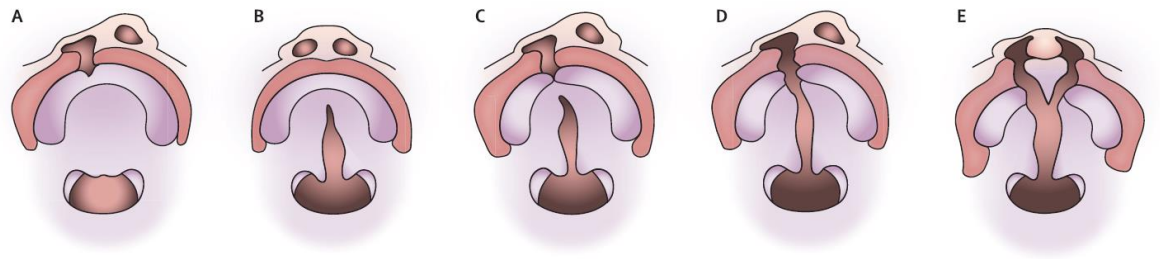

Figure 1 Phenotypes of CLP.
(A) CL and alveolus. (B) CP. (C) Incomplete unilateral CLP. (D) Complete unilateral CLP. (E) Complete bilateral CLP. (Source: Mossey *et al.*, 2009)

2.2 Epidemiology

Oral cleft (OC) is one of the most common birth defects among humans. According to the World Health Organization (WHO) in 2001, every two minutes somewhere in the world a child is born with some form of cleft, and it occurs in about 1 in every 700 live births (Mossey and Castillia, 2003). This varies according to ethnicity, with Native American Indians having the highest incidence of CL/P with 3.6 in every 1000 livebirths, followed by the Japanese with 2.1 in every 1000 livebirths (Croen *et al.*, 1998). In Caucasians, the incidence of OFC is reasonably constant with 1:800 to 1:1000 for CLP and 1:800 for CP (Fraser, 1970; Bonaiti *et al.*, 1982; Gorlin *et al.*, 2001). There is also a gender influence with CL/P being more common in males, while isolated CP is more common in females (Mossey and Little, 2002). Bilateral clefts are more severe than unilateral clefts. Fortunately, they occur less frequently, comprising only 10% of all clefts (Bender, 2000). In terms of laterality, left-sided clefts are more common than right-sided clefts and are associated with lower levels of academic performance than individuals with right-sided clefts, who showed similar performance to their unaffected classmates (Gallagher *et al.*, 2017). Table 1 summarises the clinical features of OFC.

Features	Findings
Sex	CL/P: M > F CP: F > M
Site	CL: 20% CLP: 46% CP: 33%
Laterality of CL	Unilateral CL: • Left > Right (2:1) • Unilateral > Bilateral (9:1) • Associated with CP in 68% of cases Bilateral CL: • Associated with CP in 86% of cases
Types of CL	Microform (presence of vertical groove and vermillion notching) Unilateral CL (complete or incomplete) Bilateral CL
Types of CP	CP associated with CL Isolated CP • CP (hard or soft palate) Submucous CP

Table 1 Clinical features of cleft. (Source: Chen, 2006)

2.3 Embryonic Development

Development of the orofacial structures is a complex process involving several stages, namely: cell migration, growth, differentiation, and apoptosis. These key stages of embryonic development require critical regulation.

2.3.1 Facial Development

During the 4th week of human embryonic development, neural crest cells migrate through the mesenchyme to the developing craniofacial region and form the five primitive facial processes: frontonasal process, right and left maxillary processes, and right and left mandibular processes. These facial processes containing mesenchyme, surrounded by an

epithelial barrier, merge to form the orofacial structures, as illustrated in Figure 2. Two theories exist on how these facial processes unite. One theory explains that as one facial process approximates another, the epithelial layer resorbs and the two processes "fuse" together. The other theory states that these processes correspond to mesenchymal growth centres that lead to "merging" of the processes rather than fusion (Streeter, 1948).

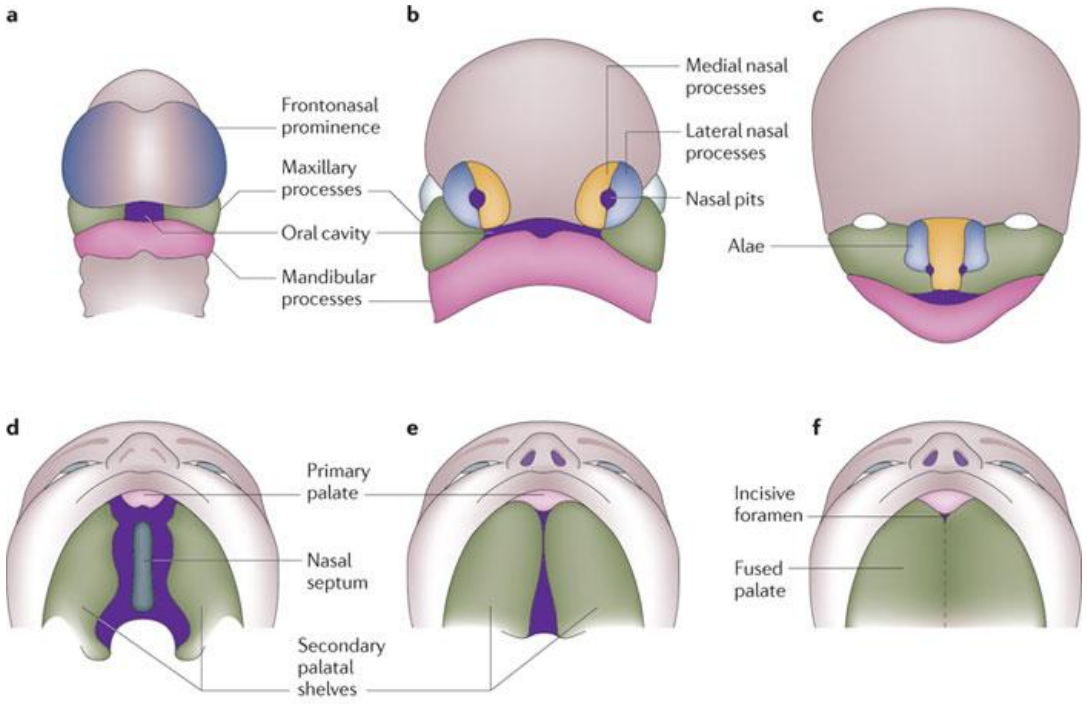

Figure 2 Development of the lip and palate.
(a) The frontonasal prominence and paired maxillary and mandibular processes surround the oral cavity. (b) The medial and lateral nasal processes develop. (c) The medial nasal processes unite to form the philtrum, primary palate, and maxillary incisors. The medial and maxillary processes unite to form the rest of the upper lip, and the medial, lateral and maxillary processes unite to form the alae of the nose. (d) The maxillary processes extend bilaterally to form the palatal shelves. (e) The palatal shelves elevate and merge to form the secondary palate. (f) This fusion creates two cavities: the oral and nasal cavities. (Source: Dixon *et al.*, 2011)

2.3.2 Lip Development

The development of the lip occurs during the 5th and 6th weeks *in utero*. The lip is formed by fusion of the maxillary processes below the lateral nasal processes. As the maxillary processes grow in a medial direction, they merge with the medial nasal processes to form

the philtrum and tissues of the upper lip. Failure of fusion of these processes may result in a CL.

2.3.3 Palate Development

Palate development, or palatogenesis, takes place at the end of the 5th week of embryonic development and consists of two parts:

- Development of the primary palate
- Development of the secondary palate

The medial nasal processes unite to form the primary palate, which in turn gives rise to the four upper incisors and the surrounding alveolar bone. The secondary palate, on the other hand, develops as a result of fusion of the maxillary processes. This union gives rise to the remaining maxillary teeth as well as the hard and soft palate.

During embryonic development, the hard palate develops as two separate halves, which extend from the anterior border of the lips to the posterior border of the uvula. The palatal shelves, on either side of the tongue, grow vertically and elevate to a horizontal position above the dorsum of the tongue (Sandy and Brown, 2002). This elevation occurs as a result of one or both of the following mechanisms:

1. Extrinsic forces – movement of the tongue, increase in the size of the mandibular prominence, lifting of the head, straightening of the cranial base, and an increase in the height of the oronasal cavity.
2. Intrinsic forces – change in the osmotic pressure, muscular and non-muscular contractions, cellular reorganisation, and vascular forces.

In normal development, the palatal shelves fuse creating a continuity in the underlying mesenchyme. For the palatal shelves to fuse, the medial epithelial seam breaks down either by apoptosis (Cuervo and Covarrubias, 2004; Vaziri Sani *et al.*, 2005) or by transformation of the epithelium into mesenchyme (Sun *et al.*, 2000; Nawshad and Hay, 2003). In 2007, Ahmed *et al.* showed that both epithelial transformation and apoptosis are necessary for fusion of the palatal halves to take place. Lack of fusion of one or more of these tissues will result in CP.

Table 2 summarises embryonic orofacial development, including the lip and palate. It clearly shows what facial processes fuse or merge together to form the corresponding facial structures.

Facial Processes	Facial Structures
Mandibular process + mandibular process	Lower lip Lower part of cheeks Other mandibular structures
Medial nasal processes + maxillary processes	Philtrum Primary palate Maxillary incisors Surrounding alveolar bone
Maxillary process + maxillary process	Upper lip Secondary palate Upper part of cheeks
Lateral + medial nasal processes + maxillary process	Ala of nose

Table 2 Orofacial development. (Source: Sandy and Brown, 2004; Cobourne and DiBiase, 2015)

2.4 Pathophysiology

Cleft lip develops because of a failure of part of the frontonasal process, medial and lateral nasal processes, and the maxillary process to unite. For a unilateral cleft of the lip to develop, the maxillary process on one side fails to unite with the merged medial nasal

processes. Whereas, for a bilateral CL to develop, the same disruption takes place in both maxillary processes. Cleft palate, on the other hand, develops either because of failure of the medial nasal processes to unite, failure of the maxillary processes to unite, or a combination of both (Moore and Persuad, 1993).

In summary, CL/P develops due to:

- Failure of fusion of facial processes
- Failure of elevation of one or both palatal shelves
- Failure of epithelial breakdown between the palatal shelves

2.5 Aetiology

Cleft lip with or without cleft palate is said to be of multifactorial or polygenic inheritance meaning that the condition is caused by both genetic and environmental factors. These genetic abnormalities and/or environmental disturbances alter the composition of the extracellular matrix (ECM), affecting cell patterning, migration, proliferation, and differentiation. This in turn may result in failure of fusion or merging of the facial processes causing an OFC to develop (Young *et al.*, 2000).

Carinci *et al.* in 2007 stated that any changes in the distribution of ECM components, cytokines, and growth factors (GF), such as fibroblast growth factor (FGF) 2 and transforming growth factor (TGF) beta-3 (TGFβ-3), may affect the regulation of the complex events that take place during cranial and orofacial development.

Extracellular matrix is especially crucial for the development of the palate. The molecules of the ECM activate GF and cytokines present in the epithelial cells and palatal mesenchyme

(Qiu and Ferguson, 1995). Therefore, any disturbances in the ECM may give rise to CP. Mutations and/or deficiencies in TGFβ-3 may also give rise to CP (Lidral *et al.*, 1998).

2.5.1 Genetic Factors

Linkage and association studies have played a major role in the identification of genes associated with the development of OFC (Adeyemo and Butali, 2017). Linkage is used to identify specific mutations that are common among all affected individuals (Teare and Barrett, 2005). This is done by comparing affected chromosomes with those of unaffected individuals. Association, on the other hand, is used to identify chromosomal segments when not much is known about the affected individual (Cordell and Clayton, 2005). Some of these identified genes include TGF beta (TGFβ) (Ardinger *et al*., 1989), MSX1 (Jezewski *et al*., 2003; Butali *et al*., 2011), and AP2 (Rahimov *et al*., 2008).

Another way of identifying the genes that cause cleft is by using mouse models with the insertion and deletion of specific genes. The three most common examples of knockouts are:

1. MSX1 (Satokata and Maas, 1994; Houzelstein *et al*., 1997)
2. TGFβ-3 (Proetzel *et al*., 1995; Kaartinen *et al*., 1995)
3. AP2 (Notolli *et al*., 1998)

Expression of these genes is involved in craniofacial development and thus, their knockout results in clefts. Mutations in MSX1 and TGFβ-3 have been shown to result in CP. Whereas, mutations in AP2 resulted in more generalised craniofacial defects including clefts.

2.5.1.1 Inheritance

The risk of inheriting CL/P increases with an increase in the severity of the phenotype, degree of kinship, and number of affected relatives. The greater the number of individuals affected, the greater the chance of inheriting CL/P (Bender, 2000). Evidence indicates a strong genetic influence on the development of NSOFC with a heritability rate of more than 90%, and if a first-degree relative is affected, the risk increases by 30 to 40 folds (Sivertsen *et al.*, 2008; Grosen *et al.*, 2010). Studies on siblings and twins have helped to clarify the inheritance pattern of NSOFC and the influence of genetic factors. The risk of having a baby with CL/P is higher among monozygotic twins (identical) than dizygotic twins (non-identical), with a rate of 40-60% and 5% respectively (Murray, 2002). Since the concordance rate among monozygotic twins is not 100%, this suggests that genetic factors alone are not the only aetiological factor responsible for the development of OFC.

2.5.2 Environmental Factors

Although the exact cause of NSOFC remains unknown, it has been associated with several risk factors. Some of the major risk factors include maternal smoking, alcohol consumption, nutritional intake, fever, medical conditions, use of medication, illicit substances, exposure to occupational hazards, obesity, and socioeconomic status. These will now be described.

2.5.2.1 Smoking

There is evidence of a significant association between maternal smoking and CL/P, especially during the first trimester. In an analysis of 4,268 cases in China, smoking was shown to be the second greatest risk factor of OFC during the first trimester of pregnancy (Meng *et al.*, 2006). Two systematic reviews and meta-analyses by Wyszynski *et al.* (1997) and Little *et al.*

(2004), based on 11 and 24 studies respectively, showed a statistically significant association between maternal smoking and NSOFC. Sabbagh *et al.* (2015) updated these systematic reviews, and ominously concluded that a 1.5-fold increase risk of NSOFC results with passive maternal smoking, which is the same risk as those that actively smoke.

There is also evidence on the intensity of smoking and whether this influences the development of CL/P. Most studies classified the intensity at two levels: low (1-9 cigarettes per day) and medium (10+ cigarettes per day) (Ericson *et al.*, 1979; Romitti *et al.*, 1999; Lorente *et al.*, 2000). Others have described three levels: low (1-10 cigarettes per day), medium (11-20 cigarettes per day), and high (21+ cigarettes per day) (Czeizel and Nagy, 1986; Khoury *et al.*, 1987). Little *et al.* (2004) analysed eight studies that had sufficient information to perform a dose-response analysis. Four of the eight studies had a weak positive dose-response relationship, and the remainder showed no relationship at all. It was concluded that there was no strong evidence to correlate the dose of cigarette smoking to the risk of OFC development.

2.5.2.2 Alcohol

Alcohol is a known teratogen that can cause a wide range of foetal defects. One of the most severe is foetal alcohol syndrome (FAS), characterised by CAs, growth retardation, and central nervous system disorders (Clarren and Smith, 1978). Cleft lip with or without cleft palate occurs in 9-18% of individuals with FAS. However, CL/P is not characteristic of this syndrome (Abel, 1998).

Measuring alcohol consumption is difficult and mainly comprises self-reporting. There are inconsistencies due to the vagueness in the type and amount of alcohol consumption recorded. Some evidence shows that even with low levels of alcohol, there is a significant

increase in the risk of developing OFC (Munger *et al.*, 1996). While other studies have found that there is no risk of developing cleft with low levels of alcohol, but increased risk with high levels (Werler *et al.*, 1991; Shaw and Lammer, 1999).

Many studies have shown consistent results when it comes to binge drinking; that is drinking five or more units of alcohol per sitting. There is an increase in the risk of a foetus developing a cleft when mothers engage in binge drinking during pregnancy (Gladstone *et al.*, 1996). A case-control study in Norway in 2008 studied 377 participants with CL/P and 196 with isolated CP. They found that when compared with women who did not drink, women who binge drink had a greater risk of having infants with CL/P (Deroo *et al.*, 2008).

2.5.2.3 Nutritional Intake

Nutritional intake during pregnancy has been identified as a factor in the aetiology of OFC (Shaw *et al*., 2006). However, the extent to which each nutrient is associated with CL/P remains unknown.

2.5.2.3.1 Folic Acid

The association between maternal intake of folic acid has been studied and the evidence is inconsistent. It is highly likely that folic acid intake varies greatly between populations, but several studies have shown that mothers who take vitamin supplements containing folic acid have a reduced risk for an infant to be born with CL/P (Shaw *et al.*, 1995; Bailey and Berry, 2005; Wilcox *et al.*, 2007). Wehby *et al.* (2013) conducted the first double-blinded randomised control trial studying the effects of high and low doses of folic acid in women who were born with a cleft or had a child with a cleft. The OC recurrence rates were 2.9% and 2.5% in the 0.4 and 4 mg of folic acid groups, respectively. The results were similar

between the two folic acid groups, suggesting that there is no clinically significant difference in the dose of folic acid and the development of CL/P. However, the trial was underpowered due the high number of withdrawals and terminations. This means that there was insufficient statistical power to detect the smallest difference in recurrence rates between the low and high dose groups.

A systematic review, updated in the Cochrane database in 2015, entitled "Effects and Safety of Periconceptional Oral Folate Supplementation for Preventing Birth Defects" concluded that folic acid can prevent neural tube defects. However, its effect on specific birth defects, such as CL/P is not clear (De-Regil *et al.*, 2015).

2.5.2.3.2 Vitamin B

Although folic acid has been the main nutritional intake investigated, other nutrients have also been studied and have been found to contribute to lowering the risk of developing OFC. After comparing the intake of mothers of infants with NSOFC to controls, Krapels *et al.* (2004) concluded that periconceptional maternal intake of vitamin B, including thiamine, niacin, and pyridoxine, significantly reduces the risk of NSOFC. However, this may be because of confounding factors, such as folic acid, or simply because these mothers who are exposed to these vitamins live healthier lifestyles with a dietary intake high in vitamin B. Vitamin B contributing to the prevention of NSOFC has also been confirmed in animal experiments. In one study by Schubert *et al.* (2002), murine strains were given high levels of vitamin B, and as a result, these strains had a lower incidence of OFC.

2.5.2.3.3 Vitamin A

Retinol, also known as vitamin A, is commonly used to treat various dermatological conditions and has been associated with several congenital malformations, one of which is foetal retinoid syndrome (FRS). One of the craniofacial abnormalities that may be present in infants with FRS is CL/P. The teratogenicity of vitamin A from both diet and supplements was studied in a prospective cohort study consisting of 22,748 pregnant women in Boston. The authors concluded that high dietary intake of vitamin A is potentially teratogenic. It was also estimated that about 1 in every 57 infants exposed to vitamin A had a malformation, including OC (Rothman *et al.*, 1995).

Teratogenicity of retinoids has been linked to oral intake, but it may be teratogenic when used topically during pregnancy, as well. A systematic review and meta-analysis by Kaplan *et al.* (2015) found no increase in the rates of major congenital malformations (MCMs), spontaneous abortions, low birthweight, or premature delivery when retinoids were used topically. This is reassuring to women who have been exposed to topical retinoids when they were not aware of their pregnancy. However, due to the low statistical power of the study, topical retinoids should be cautiously prescribed during pregnancy (Kaplan *et al.*, 2015).

2.5.2.3.4 Zinc

Antioxidants, such as zinc, have been studied and seem to be associated with lower levels of OFC (Tamura, *et al.*, 2005; Wallenstein, *et al.*, 2013). However, a true causal relationship cannot be deduced as zinc concentration in plasma is affected by a number of external

factors. Some of which include exercise, trauma, infection, inflammation, as well as the time when the blood sample was taken and the time of the last meal.

2.5.2.4 Infection and Fever

Exposure to infection and fever during pregnancy has been suggested to have an association with a higher risk of CL/P. Wang *et al.* (2009) concluded that a history of fever or cold during the first trimester of pregnancy is associated with an approximate threefold increase of OFC. This causal finding is consistent with two other previous studies (Hakosalo and Saxen, 1971; Zhang and Cai, 1993). A systematic review (17 papers) on adverse outcomes for infants where mothers had a history of fever during pregnancy, confirmed that there is an association between gestational fever and congenital anomalies, especially neural tube defects, congenital heart defects, and OC (Dreier *et al.*, 2014).

2.5.2.5 Medical Conditions and Related Medication

This dissertation will focus on the effects of medication, specifically macrolides, antifungals, and antivirals/ antiretrovirals, taken during pregnancy on CL/P. Therefore, most of the information related to this heading will be presented later in the discussion and briefly in the literature review.

2.5.2.5.1 Hyperthyroidism

A number of studies have investigated the effects of antithyroid drugs on the risk of congenital anomalies, one of which is CL/P. Hyperthyroidism affects 0.2%-2.7% of pregnancies worldwide (Li *et al.*, 2015). Antithyroid drugs, such as propylthiouracil (PTU), methiamazole (MMI), and carbimazole (CMZ), are considered the drugs of choice for hyperthyroidism during pregnancy, although it is well known that these drugs do have a

tendency to cross the placenta and influence foetal development. A meta-analysis (eight studies) showed that when pregnant women are exposed to PTU or MMI/CMZ there is an increased risk of their children developing congenital anomalies (Li *et al.*, 2015). These results were consistent with a systematic review published the year before (Laurberg and Andersen, 2014).

2.5.2.5.2 Asthma

Asthma is another common condition that affects pregnant women with approximately 8.4-8.8% reporting asthma (Kwon *et al*., 2006). Asthma is treated with ant-inflammatory drugs, such as corticosteroids, mast cell stabilisers, and leukotriene modifiers, as well as bronchodilators such as β_2-adrenergic agonists, anticholinergic agents, and theophylline. Asthmatic women are at a greater risk of adverse pregnancy outcomes (Demissie *et al*., 1998; Tamasi *et al*., 2006). Despite these known facts, the National Education and Prevention Program (NAEEP) still recommends the continuation of asthma medication throughout the course of the pregnancy (NAEEP, 2005).

Oral corticosteroids have been found to contribute to the development of CL/P when taken during the first trimester (Park-Willie *et al.*, 2000; Carmichael *et al.*, 2007), as do bronchodilators. In a study performed on pregnant mice and rabbits, bronchodilators were administered to assess whether these drugs influenced the development of CP. They concluded that there is a statistically significant association between maternal bronchodilator use and CL/P (Szabo *et al.*, 1975; Shibata *et al.*, 2000). A similar case control study conducted on humans confirmed the same finding (Munsie *et al.*, 2011).

2.5.2.5.3 Obesity

Obesity is a major health problem and an economic burden. Pre-pregnancy maternal obesity can influence both the mother and the child. Mothers may develop gestational diabetes and hypertensive disorders, such as preeclampsia. Infants of obese mothers on the other hand, are at increased risk of birth complications, perinatal death, and congenital anomalies. A systematic review and meta-analysis assessed the relationship between maternal obesity and the risk of congenital anomalies. Obese mothers are at significantly increased risk of having an infant with CLP or isolated CP when compared with mothers of recommended body mass index (BMI), but this was not the case for isolated CL (Stothard *et al.*, 2009).

2.5.2.5.4 Diabetes Mellitus

Pregnant women who are both obese and diabetic are three times more likely to have an infant with craniofacial anomalies (Moore *et al.*, 2000) including OFC (Spilson *et al.*, 2001). In a cross-sectional observational study of 325 mothers with a child with CLP, the prevalence of diabetes mellitus (DM) was 27%, which is significantly higher than the general Brazilian population of 7.6%. In addition to its association with CLP, DM also increases the risk of other craniofacial anomalies (Trindade-Suedam *et al.*, 2016).

2.5.2.6 Illicit Substances

The prevalence of prenatal illicit substance abuse in the United States of America ranges from 6.2% to 12.4% (Arria *et al.*, 2006; Bauer *et al.*, 2005; Behnke *et al.*, 2001; El-Mohandes *et al.*, 2003). Determining the true association between maternal drug abuse and adverse foetal outcomes is challenging because drug abuse is usually accompanied by other confounding factors, such as smoking and alcohol. Furthermore, the sample size is usually

small because not everyone recruited to a study will admit to taking part in what is considered an illegal behaviour. Fear of judgement and prosecution underestimates the true outcome of these studies.

Mothers who use illicit drugs during their pregnancy are three times more likely to give birth to children with CL/P in association with other congenital anomalies, than mothers who do engage in these recreational habits (Trindade-Suedam *et al.*, 2016). Based on the evidence available, marijuana does not appear to be associated with major congenital anomalies (Lee, 1998; Witter and Niebyl, 1990). However, cocaine seems to have a major influence on congenital anomalies. Using data from the National Birth Defects Prevention Study, an association was found between periconceptional cocaine use and the development of CP (van Gelder *et al.*, 2009). Cocaine is known to cause vasoconstriction and sudden hypertension. This in turn interrupts blood flow to certain tissues of the embryo, leading to CP formation. It can also be speculated that certain illicit substances when taken together can potentiate their effects in causing congenital anomalies to develop.

No relevant animal studies have been carried out investigating cocaine. However, marijuana use during pregnancy has been shown to cause neural tube defects in rabbits and hamsters, which is not the case in human studies (Geber and Schramm, 1969).

2.5.2.7 Occupational Hazards

Since the number of women in the workforce has been increasing substantially, maternal exposure to occupational hazards is a highly relevant and important factor to study. Unfortunately, little research has been undertaken to clearly conclude whether exposure to occupational hazards is a potential risk factor for CLP.

2.5.2.7.1 Solvents

Studies by Cordier *et al.* (1992) and Lorente *et al.* (2000) found an association between the occurrence of CL/P with maternal occupations, such as janitors or cleaners. This is most likely because these mothers were exposed to solvents. Maternal exposure to solvents is linked to MCMs, some of which include urinary malformations, male genital malformations and OFC. Nurses, laboratory workers and hairdressers were also found to be affected (Garleantezec *et al.*, 2009). Of the studies on this subject, one did not find a greater risk of CL/P with maternal occupational exposure to solvents (Shaw *et al.*, 2003).

2.5.2.7.2 Pesticides

Pesticide exposure has also been investigated, and a systematic review and meta-analysis by Romitti *et al.* (2007) concluded that maternal exposure to pesticides has a small risk to the development of OFC. In 2014, Yang *et al.* studied the effect of agricultural pesticides on clefts and confirmed that there is a risk of OFC with maternal exposure to pesticides.

2.5.2.7.3 Metals, Dust, Gases, and Fumes

Not much evidence is available on maternal occupational exposure to metals, dust, gases and fumes. One study concluded that there is an association between metals and OFC (Hao *et al.*, 2015). Spinder *et al.* (2017), on the other hand, found no significant association between the two. In addition, no association was found between maternal occupational exposure to gases and fumes and OFC (Spinder *et al.,* 2017). However, the authors did find a causal relationship with maternal occupational exposure to dust.

2.5.2.8 Socioeconomic Status

Womersley and Stone (1987) studied the effects of socioeconomic status in Greater Glasgow, Scotland and the risk of developing CL/P. They concluded that OFC were more common in socioeconomically deprived areas of the city, where residents had high unemployment rates and the majority were unskilled workers. Other epidemiological studies have reported similar findings: one in Wales (Durning *et al.*, 2007) and another in California (Carmichael *et al.*, 2009).

2.6 Classification

Several classification models for CL/P have been formulated over the years. Listed below are the models in chronological order:

- Davies and Ritchie (1922)
- Veau (1931)
- Fogh-Andersen (1942)
- Kernahan and Stark (1958)
- Santiago (1969)
- WHO (Bezroukov, 1979)
- Kriens (1989)

In the United Kingdom, the Kriens model, which utilises the LAHSAL code, has been chosen as the preferred classification method for clefts. The LAHSAL code, shown in Figure 3, is based on the striped Y diagrammatic classification. It splits the relevant parts of the oral cavity into six parts:

- Right lip

- Right alveolus
- Hard palate
- Soft palate
- Left alveolus
- Left lip

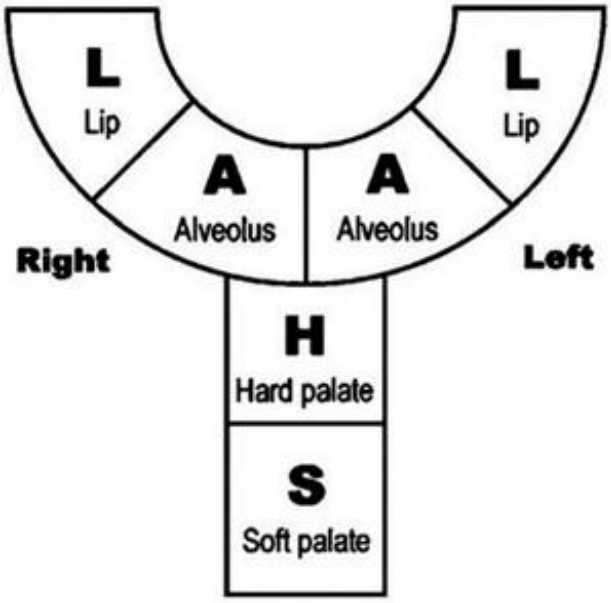

Figure 3 LAHASL code for classifying clefts. (Source: Kriens, 1989)

The LAHSAL code is used with direct visualisation of the mouth and face. For example, the first character “L” on the left corresponds to the patient's right lip, and the last character “L” on the right corresponds to the patient's left lip. The code also indicates whether there is a complete cleft, written with an uppercase letter, or an incomplete cleft, written with a lowercase letter, or no cleft at all.

2.7 Diagnosis

Cleft lip with or without cleft palate can be detected with routine ultrasound examination (Clementi *et al*., 2000; Chmait *et al*., 2002). Chmait *et al*. (2002) showed that 100% of CL

cases and 90% of CP cases were identified with two-dimensional ultrasound with three-dimensional ultrasound as an adjunct. Cases of CLP can be detected as early as 11 weeks *in utero* (Gullino *et al*., 2006). Submucous CP, on the other hand, is difficult to detect due to mucosa covering the cleft. It is diagnosed very late at a mean age of 4.9 years (Reiter *et al*., 2011). These clefts are usually noticed by reported symptoms and physical examination. Common symptoms include hypernasal speech, Eustachian tube dysfunctions with conductive hearing loss, and nasal reflux when feeding (Reiter *et al*., 2011). Velopharyngeal impairment, failure of the soft palate to create a seal between the oral and nasal cavity, is diagnosed by at least one of the following diagnostic procedures: nasoendoscopy or videofluoroscopy (Nagarajan *et al.*, 2009).

The National Health Service (NHS) Foetal Anomaly Screening Programme (FASP) offers screening to all eligible pregnant women. The screening process consists of two scans. The first scan, which is done between weeks 10 to 14 of pregnancy, is preformed to detect Down's syndrome, Edward's syndrome and Patau's syndrome. The second scan, which is recommended by the National Institute of Health and Care Excellence (NICE) guidelines, is to detect foetal anomalies and is performed between 18 weeks 0 days to 20 weeks 6 days of pregnancy.

Table 3 lists the medical conditions that are screened in England and their corresponding detection rates.

Conditions	Detection Rate (%)
Anencephaly	98%
Open spina bifida	90%
Cleft lip	75%
Diaphragmatic hernia	60%
Gastroschisis	98%
Exomphalos	80%
Serious cardiac anomalies: • Transposition of the Great Arteries (TGA) • Atrioventricular Septal Defect (AVSD) • Tetralogy of Fallot (TOF) • Hypoplastic Left Heart Syndrome (HLHS)	50%
Bilateral renal agenesis	84%
Lethal skeletal dysplasia	60%
Edward's syndrome (Trisomy 18)	95%**
Patau's syndrome (Trisomy 13)	95%**

Table 3 Conditions screened as a minimum in England.
** Detections rates will be reviewed once sufficient data is received following implementation of screening as part of the combined screening strategy.
(Source: NHS Foetal Anomaly Screening Programme Handbook, 2018)

2.8 Treatment

Orofacial cleft is a complex disorder that requires long term multidisciplinary care and early intervention. The multidisciplinary team usually consists of:

- Ear, nose, and throat specialists
- Paediatric dentists
- Orthodontists
- Oral and maxillofacial surgeons
- Speech therapists
- Nutritionists

- Psychologists
- Geneticists

In the United Kingdom, cleft services have been based around a centralised service for the last two decades (Sandy, 2019). The cleft team aims to provide cleft patients and their families with the appropriate care immediately after diagnosis. This usually involves a tailored care plan to meet each affected child's needs. A standard care plan timetable for CL/P patients is shown in Table 4.

Age	Intervention
Birth to 6 weeks	Feeding assistance Support for parents Hearing tests Paediatric assessment
3 to 6 months	CL surgery
6 to 12 months	CP surgery
18 months	Speech assessment
3 years	Speech assessment
5 years	Speech assessment
8 to 12 years	Alveolar bone graft
12 to 15 years	Orthodontic treatment Monitoring jaw growth

Table 4 Chronology of CL/P care plan. (Source: NHS Treatment: Cleft lip and palate, 2016).

Orofacial clefts are associated with maxillary growth restriction. This is usually due to scarring as a result of the lip and palate repair surgeries performed early during childhood. Therefore, individuals with CL/P usually require orthognathic surgery when growth has ceased to correct the anteroposterior discrepancy in the maxilla and mandible. Dental anomalies are also commonly present in individuals with CL/P. These anomalies include hypodontia, either developmentally absent or extracted for bone grafting purposes,

microdontia, supernumeraries, fusion, ectopic eruptions, taurodontism, and anterior crossbites (Aizenbud *et al.*, 2011). For this reason, orthodontic treatment is usually needed in CL/P patients.

2.9 Hierarchy of Evidence

Evidence-based practice is defined as "the integration of the best research evidence with clinical expertise, patient values and patient circumstances" (Straus *et al.*, 2011). In other words, it is finding the evidence available and using it to make clinical decisions. Although experience and opinions play a role, the focus should be on evidence-based practice.

Straus *et al.* (2011) have written up five steps to practice evidence-based medicine, namely:

1. Formulating the right clinical question
2. Finding the best evidence
3. Critically appraising the evidence
4. Integrating critical appraisal with clinical practice and the patient
5. Evaluating effectiveness

A hierarchy of evidence, illustrated in Figure 4, ranks studies according to the methodology of the design, validity, and whether the conclusions can be applied to clinical practice.

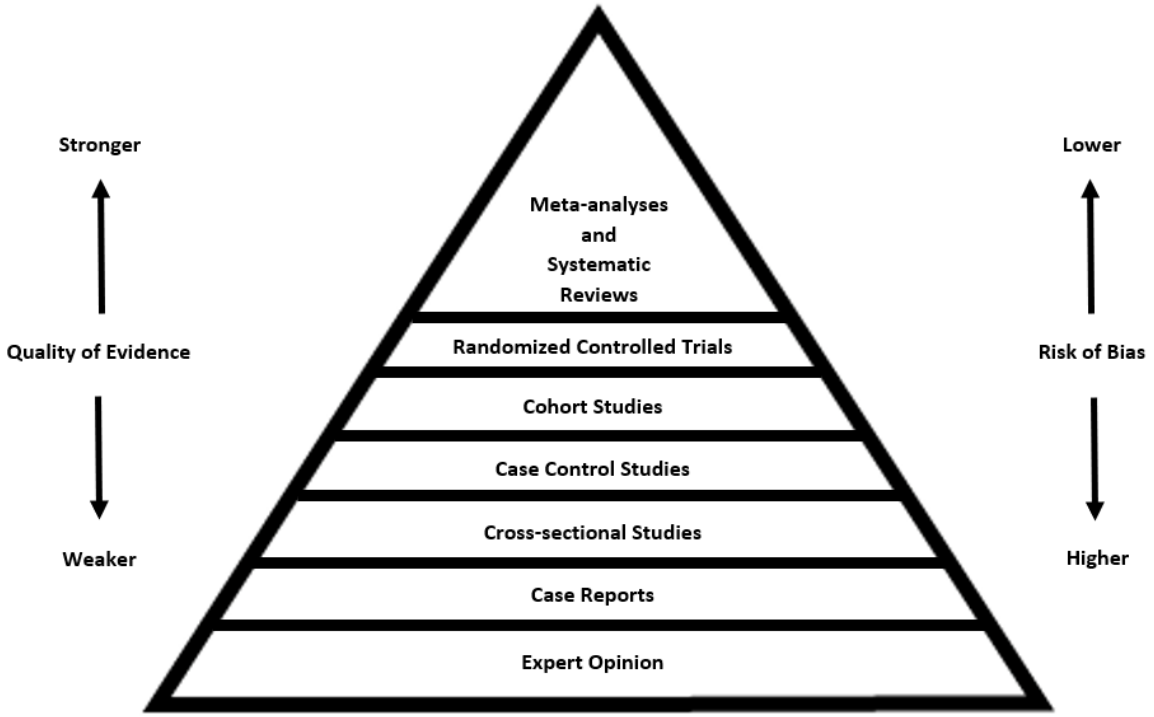

Figure 4 Hierarchy of evidence. (Source: Guyatt *et al*., 1995)

Randomised controlled trial are the gold standard for comparing different interventions and are the best in determining a causal relationship. This study design is used to minimise bias and reduce the risk of systematic errors. The factors that make this study design robust and able to provide strong evidence are (Sibbald and Roland, 1998):

- Randomisation
- Blinding
- Groups are treated equally except for the intervention
- Intention to treat analysis is applied

Unfortunately, randomised controlled trials cannot be applied to every clinical situation due to ethical reasons. Other drawbacks include difficulty in randomisation or recruitment of subjects, and they are more expensive and time consuming to run than other study designs.

Systematic reviews and meta-analyses are usually the first studies to be searched when trying to answer a question of interest due to their high level of evidence. This is because they have been written based on a standard methodological process and have been critically appraised, as well. Being situated at the top of the pyramid means that very few articles are available to answer the question at hand, and therefore such reviews may well include articles of lower levels of evidence.

2.10 Systematic Review Overview

A systematic review is a collection of all current evidence on a specific topic, answering a clearly formulated research question through critical appraisal (Antman *et al*., 1992; Oxman and Guyatt, 1993). Systematic reviews provide healthcare workers with a summary of all relevant studies to date. Over 5000 systematics reviews are stored in the Cochrane Database of Systematic Reviews, which is part of the Cochrane Library (Cochrane, 1972). This open access electronic database is a great source of information for healthcare workers to make the best clinical decisions based on the most reliable evidence available. Many systematic reviews contain meta-analyses, which use statistical methods to summarise the results of individual studies (Glass, 1976).

The key characteristics of a systematic review include (Higgins *et al*., 2017):

- A clearly stated set of objectives with pre-defined eligibility criteria
- An explicit, reproducible methodology
- A systematic search that attempts to identify all relevant studies
- An assessment of the validity of the findings of the included studies
- A systematic presentation and analysis of the findings of the included studies

Systematic reviews and meta-analyses are becoming increasingly important today in the field of healthcare. There is so much published information that most healthcare providers do not have the time nor the skills to read and analyse the information themselves. Systematic reviews and meta-analyses summarise all the information available and present it in a single paper. Systematic reviews are up to date, present important clinical practice guidelines, and provide justification for the need for further research on the topic at hand.

2.11 Systematic Reviews on Medication During Pregnancy and CL/P

To have an idea of the literature available on the effects of medication during pregnancy on CL/P, a comprehensive search was performed. The following electronic databases were searched: MEDLINE, EMBASE, and the Cochrane library, using the keywords “cleft”, “medicine”, and “systematic review”. Six systematic reviews were obtained:

1. Veroniki *et al.*, 2017. Comparative safety of anti-epileptic drugs during pregnancy: A systematic review and network meta-analysis of congenital malformations and prenatal outcomes.
2. Alsaad *et al.*, 2015. First trimester exposure to topiramate and the risk of oral clefts in the offspring: A systematic review and meta-analysis.
3. Jentink *et al.*, 2010. Intrauterine exposure to carbamazepine and specific congenital malformations: Systematic review and case-control study.
4. Chi *et al.*, 2015. Safety of topical corticosteroids in pregnancy (review).
5. Goldberg *et al.*, 2015. Exposure to nitrofurantoin during early pregnancy and congenital malformations: A systematic review and meta-analysis.

6. Murphy *et al.*, 2013. The risk of congenital malformations, perinatal mortality and neonatal hospitalisation among pregnant women with asthma: A systematic review and meta-analysis

The six systematic reviews are summarised in Table 5 and described in greater detail below.

2.11.1 Anti-Epileptics

Pregnant women with epilepsy are often prescribed anti-epileptic drugs (AED) to manage their symptoms. However, not much is known about the safety of periconceptional use of AED. Therefore, the aim of the systematic review by Veroniki *et al.* (2017) was to compare the risk of congenital malformations of infants exposed to AED *in utero* to those not exposed. MEDLINE, EMBASE, and Cochrane CENTRAL were searched. These studies compared mono- or poly-therapy AED exposed infants to infants not exposed to AED, and the primary outcome they investigated was the incidence of MCMs, including CL/P. From the 96 eligible studies they looked at, the authors concluded that monotherapies (*e.g.* ethosuximide, primidone, topiramate, phenobarbital, phenytoin and valproate) and polytherapies (*e.g.* phenobarbital plus phenytoin plus primidone, phenytoin plus primidone, carbamazepine plus phenobarbital, and carbamazepine plus valproate) were both associated with a higher rate of CL/P than the controls.

2.11.1.1 Topiramate

Topiramate (TPM), one of the newer AED, has been approved for the treatment of epilepsy. It is also used to treat migraines and sleep and psychiatric disorders, as well as acting as an appetite suppressor. While some studies have shown an increased risk of OFC, equally, other studies suggest the contrary. A meta-analysis was conducted on all studies reporting

mothers exposed to TPM during pregnancy (Alsaad *et al.*, 2015). Six articles met the inclusion criteria and this study provided strong evidence that first trimester exposure to TPM is associated with a six-fold increased risk of OFC. This conclusion implies that women of child-bearing age taking TPM should be carefully monitored, and pregnant women should be informed about the teratogenic risks associated with this therapeutic agent.

2.11.1.2 Carbamazepine

Carbamazepine is one of the most common AED used among pregnant women in Europe. Several studies have evaluated the risk of MCMs associated with carbamazepine. However, because of the small sample size of each individual study, the statistical power was not sufficient to detect whether there was a risk of specific congenital malformations. Therefore, the aim of the systematic review by Jentink *et al.* (2010) was to identify the risk of specific malformations associated with first trimester exposure to carbamazepine. PubMed, EMBASE, Web of Science, and the EUROCAT Antiepileptic Study Database were searched. From the eight studies they included, the authors concluded that there is a 3.3% risk of congenital malformations, with a significantly lower risk of CL/P with carbamazepine monotherapies than with other AED, excluding valproic acid.

2.11.2 Topical Corticosteroids

Topical corticosteroids are the most commonly prescribed dermatological treatment. However, not much evidence is available about their safety when used during pregnancy. Chi *et al.* (2015) updated the 2009 systematic review on the safety of topical corticosteroids during pregnancy. MEDLINE, EMBASE, and LILACS were searched. Seven additional observational studies were added to the original seven making a total of 14 studies

investigated. They concluded that there is no association between maternal exposure to topical corticosteroids and OFC.

2.11.3 Nitrofurantoin

Urinary tract infections are one of the most common conditions that occur during pregnancy, and if not treated, may result in morbidity for both the mother and her foetus. Penicillin is the treatment of choice. However, because of the increasing resistance to penicillin, nitrofurantoin is the next commonly prescribed alternative. Unfortunately, the safety of nitrofurantoin use during pregnancy remains controversial. The recent systematic review by Goldberg *et al.* (2015) searched Medline and EMBASE and the authors included eight studies fitting the inclusion criteria. They concluded that there was no significant association between foetal exposure to nitrofurantoin during the first trimester of pregnancy and OFC. Therefore, it was concluded that nitrofurantoin can be safely used during pregnancy when resistance to penicillin is evident.

2.11.4 Bronchodilators and Inhaled Corticosteroids

Asthma is a common condition reported in pregnant women and is usually treated with bronchodilators or inhaled corticosteroids. The first systematic review and meta-analysis published on the effects of asthma during gestation was in 2011 (Murphy *et al.*, 2011). Two years later, an updated meta-analysis was published focusing more on specific congenital malformations, including CL/P (Murphy *et al.*, 2013). After extracting the data and analysing the results from sixteen studies, the authors concluded that asthma indeed was associated with a significantly increased risk of congenital malformations, specifically CL/P with an increased risk of 30%. Oddly, however, the use of bronchodilators and inhaled

corticosteroids were not the cause of the congenital malformations that resulted. Due to the limited number of studies available, the true association between maternal asthma and congenital malformations remains unclear. Maternal asthma has been associated with low birthweight and preterm delivery, which increases the risk of congenital malformations (Murphy *et al.,* 2011).

This current review of the literature has shown that no systematic analysis of the available evidence has to date been undertaken to assess the impact of foetal exposure to the maternal intake of antimicrobials, other than penicillin, on CLP. The aim of this study was therefore to systematically review the evidence from published studies on maternal exposure to macrolides, antifungals, and antivirals/ antiretrovirals on the risk of developing NSOFC.

Systematic Review	Databases Searched	Exposure	Outcome	Number of studies	Conclusion
Veroniki *et al.*, 2017	MEDLINE EMBASE Cochrane	AED	Congenital malformations	96	AEDs are associated with CL/P
Alsaad *et al.*, 2015	MEDLINE EMBASE Web of Science	TPM	OFC	6	TPM is associated with OFC
Jentink *et al.*, 2010	PubMed EMBASE Web of Science	Carbamazepine	Congenital malformations	8	Carbamazepine is associated with lower risk for CL/P
Chi *et al.*, 2015	MEDLINE EMBASE LILACS Cochrane	Topical corticosteroids	Congenital malformations	14	Topical corticosteroids are not associated with OFC
Goldberg *et al.*, 2015	MEDLINE EMBASE	Nitrofurantoin	Congenital malformations	8	Nitrofurantoin is not associated with OFC
Murphy *et al.*, 2013	MEDLINE EMBASE Cochrane	Bronchodilators and inhaled corticosteroids	Congenital malformations, neonatal complications, perinatal mortality	16	Bronchodilators and inhaled corticosteroids were not associated with CL/P

Table 5 Summary of systematic reviews on medication during pregnancy and CL/P.

3.0 MATERIALS AND METHODS

3.1 Overview

A systematic review is a thorough and detailed critical appraisal of the literature extant on a specific topic answering a clearly focused question (Moher *et al*., 2007). Systematic reviews are considered to be the second best source of research evidence after a meta-analyses, and they are particularly important in evidence-based dentistry, as they are in other fields of medicine (Straus *et al.*, 2011). A systematic review requires a protocol that defines the study aims, objectives and expected outcomes. The design of the study is clearly critical for the systematic review.

3.2 Aim

The aim of this systematic review is to investigate if there is any association between the occurrence of NSOFC and foetal exposure to macrolides, antifungals, and antivirals/ antiretrovirals *in utero*.

3.3 Objectives

- To identify all relevant studies published to date on the effect of *in utero* exposure to antimicrobials on NSOFC and to analyse the evidence.
- To provide information for future studies seeking to investigate and clarify the risks of maternal intake of antimicrobials during pregnancy in relation to NSOFC.
- To increase awareness among women of child-bearing age worldwide on the effects of antimicrobials on their unborn infant.

3.4 Null Hypothesis

The null hypothesis states that maternal exposure to either macrolides, antifungals, or antivirals/ antiretrovirals has no effect on the development of NSOFC.

3.5 Materials and Methods

A systematic review comprises a methodology which searches the literature, identifies, and selects relevant studies, and appraises and synthesises the evidence. The methodology is specific, reproducible, and aims to minimise bias. After formulating a question and defining the aims and objectives of the review, it is important to define the inclusion and exclusion criteria to select relevant articles.

3.5.1 Inclusion Criteria

The inclusion criteria for this study were:

- Infants with NSOFC
- Mothers exposed to macrolides, antifungals, and/or antivirals/ antiretrovirals *in utero*

3.5.2 Exclusion Criteria

The exclusion criteria for this study were:

- Foreign language studies
- Syndromic OFC
- Animal studies
- Books
- Letters

- Conference papers
- Literature reviews
- Case reports and case series

A systematic review is more comprehensive than a simple literature review in that it may include both published and unpublished literature. The inclusion of unpublished literature, also known as “grey” literature, adds strength to a systematic review as it is often more current than other published literature and is less likely to have publication bias. Several preliminary attempts to identify grey literature proved fruitless and it was disappointing that even much of the other published work was unable to provide raw data for further analysis.

3.5.3 Literature Search

The literature search is the major component of any systematic review process. It is a complex process that requires searching different databases. Training to undertake this systematic review comprised a four-day short course in June 2018 “Systematic Reviews and Meta-Analysis”, held at the Department of Population Health Sciences in the Medical School, University of Bristol. Access to the literature and articles was available online and from the library in the University of Bristol. Direct support was also available and freely given by the librarians.

The following electronic databases were searched: MEDLINE, Embase, and the Cochrane Library using exploded MeSH headings and text words (Appendix A). To identify terms related to antimicrobial exposure, the following search terms were combined with OR:

- antibiotic* or macrolide* or azithromycin* or clarithromycin* or erythromycin* or fidaxomicin* or telithromycin*

- antigungal* or fluconazole* or diflucan* or griseofulvin* or gris peg* or fulvicin pg* grifulvin v* or itraconazole* or sporanox* or ketoconazole* or nizarol* or terbinafine* or lamisil*
- antiviral* or amantidine* or symmetrel* or rimantadine* or flumadine* or oseltamivir* or tamiflu* or zanamivir* or relenza*

Relevant papers were identified by searching subject headings and keywords for “cleft lip”, “cleft palate”, and “orofacial cleft”. Both exposure and outcome searches were combined with AND, and the search was limited to human studies only.

3.5.4 Screening

When the search was completed, all titles and/or abstracts were screened. After the initial screening, many of the articles were rejected because of the defined initial inclusion and exclusion criteria. A full text of all the identified articles were then retrieved from the library e-journals and read in more detail. Fortunately, all articles were available online and there was no need to request an inter-library loan on behalf of the university. Papers that could not be excluded based on the initial review, were assessed by a second reviewer (NH), and any disputes were resolved by a third reviewer (AI). Duplicate articles from the different electronic databases were removed. Moreover, reference lists of included articles were hand searched for additional studies that were not identified in the initial search.

3.5.5 Data Extraction

Once all the relevant articles were identified, an electronic data extraction form was constructed to ensure that all relevant information was obtained from each of the studies included. The data extraction form that was used for this systematic review is a self-

modified version of the data extraction template provided online in the Cochrane Public Health Group. The form includes details of study and participant characteristics, setting, results, etc. (Appendix B). Where results from a single study were reported in multiple publications, data was extracted from the latest published article with the longest time interval from the initial publication.

3.5.6 Quality Assessment

No one method exists to assess quality of a study as each has its own strengths and weaknesses (Rethans *et al*., 1996). However, the majority agree on two minimum requirements:

- Selection of the appropriate study design to answer the research question
- Assessing risk of bias

Two resources used to assess the quality of studies included in this systematic review are the Critical Appraisal Skills Programme (CASP) (CASP, 2018) and the ROBINS-I risk of bias tool (Sterne *et al*., 2016).

3.5.6.1 Critical Appraisal

Sanderson *et al.* (2007) suggested a critical appraisal should include the following:

- Appropriate study design selection
- Appropriate selection of participants
- Appropriate measurement of variables
- Appropriate control of confounding factors

The online CASP was used to assess the quality of the studies included in this review, individually. The articles that were identified from the search were all observational studies, comprising of cohort and case control studies. A specific quality assessment checklist for the two different types of study designs were downloaded from CASP (Appendix C).

3.5.6.2 Risk of Bias

The Cochrane "Risk of Bias" tool was used to assess the level of bias in the systematic review. There are three tools that can be used:

- RoB 2.0 tool (revised tool for risk of bias in randomised trials)
- ROBINS-I tool (risk of bias in non-randomised studies of intervention)
- robvis (visualisation tool for risk of bias assessments in a systematic review)

As the articles included in this review were all non-randomised studies, the ROBINS-I tool was used to assess the risk of bias. There are seven domains to assess:

1. Bias due to confounding
2. Bias due to selection of participants
3. Bias in classification of interventions
4. Bias due to deviations from intended interventions
5. Bias due to missing data
6. Bias in measurement of outcomes
7. Bias in selection of the reported result

There are five risk of bias judgments to choose from. Table 6 summarises each.

Judgement	Interpretation
Low risk of bias	The study is comparable to a well performed randomised trial.
Moderate risk of bias	The study is sound for a non-randomised study but is not comparable to a well performed randomised trial.
Serious risk of bias	The study has some important problems.
Critical risk of bias	The study is too problematic to provide any useful evidence.
No information	No information on which to base a judgement about risk of bias.

Table 6 Interpretation of domain-level and overall risk of bias judgements in ROBINS-I. (Source: Sterne *et al.*, 2016)

To produce the risk of bias figures, the data from ROBINS-I was uploaded on robvis (McGuinness, 2019). This web application creates two charts:

- "Traffic light" plot of the domain-level judgements for each study
- Weighted bar plot of the distribution of risk of bias judgements within each domain

These charts are illustrated in Figures 5 and 6 (vide infra 4.4).

3.5.7 Data Synthesis

The data extracted from the literature can either be presented as a narrative review and/or a statistical review or meta-analysis. A meta-analysis usually includes numerical and graphical presentations of the data. If the studies included in a review are extremely heterogenous, it is difficult to present the data as a meta-analysis and summarising the data with a narrative approach is more appropriate.

The data in this review was collated and grouped according to:

- Exposure (*i.e*. macrolides, antifungals, and antivirals/ antiretrovirals)
- Outcome (*i.e*. CL/P, CL, CP, and CLP)

- Time of exposure *in utero* (*i.e*. periconceptional and entire pregnancy)

3.5.8 Meta-Analysis

The data collected in this study were summary statistics based on a binary response (*e.g.* presence or absence of a cleft). A random effects model was used for the meta-analysis to obtain summary estimates of the effect of antimicrobial (macrolides and antifungals), taken by the mother either periconceptually or during the entire pregnancy, on the odds of having a child with a cleft (either CL/P, CL, CP or CLP). Of the 17 papers identified in the review only eight provided sufficient data to be included in the meta-analysis. In order to be included the studies had to report the following: sample size, Odds Ratio (OR) (or the Adjusted Odds Ratio (AOR), which is an odds ratio adjusted for other predictors in the model, or Prevalence Odds Ratio (POR), which is the prevalence at a point in time), 95% confidence intervals (CI) of the OR (including AOR and POR), or provide sufficient statistics to enable these values to be calculated. There was insufficient data available on antivirals and antiretrovirals and these are described using a narrative synthesis of the relevant papers.

A random effects model was used as the assumption was that each study was measuring a true, study specific effect, not necessarily from the same population, but different populations from around the world. The advantage of a random effects over a fixed effects models is that very large studies are not given undue weight to the exclusion of the smaller studies.

The analysis was stratified by antimicrobial, cleft phenotype, and time of the antimicrobial exposure during pregnancy and is presented as forest plots. Bias was also assessed using contour enhanced funnel plots where contour lines aid the assessment of statistical significance of study estimates.

3.5.8.1 Forest Plots

Forest plots are graphs used in meta-analyses to display the findings of the individual studies included in the systematic review as well as the overall summary of results to a particular outcome (Schriger *et al*., 2010). The results are usually weighted according to the statistical power of the study, and this is represented by a box. Studies with larger sample sizes and smaller CI, are indicated with a larger box and contribute greater to the pooled analysis. Whereas studies of lower power are indicated by a smaller box. A standard format of forest plots usually consists of two columns. The column on the left lists the studies, and the column on the right plots the measures of effect, such as the OR.

3.5.8.2 Funnel Plots

Introduced by Light and Pillemer in 1984, funnel plots are scatterplots of effect estimates plotted against the measure of study size, which is usually the standard error. They are commonly used in meta-analyses to detect publication and other types of biases. The effect estimates from smaller studies are scattered at the bottom of the pyramid and larger studies taper towards the top. A symmetrical funnel plot usually implies the absence of bias. However, asymmetry does not always indicate reporting bias but can also be a result of heterogeneity or simply by chance (Sterne *et al*., 2011). Funnel plots can be improved by the addition of contours of statistical significance, which facilitate interpretation of the funnel plot and determine whether the cause of asymmetry is likely due to publication bias or not (Palmer *et al*., 2008).

4.0 RESULTS

4.1 Studies Included in the Systematic Review

The search strategy yielded 165 potentially relevant papers for inclusion according to the MeSH headings and text words entered. After screening, only 25 papers were identified as meeting the inclusion criteria. Duplicates were removed and only 15 papers remained. The full-text articles were retrieved to assess eligibility. Of the 15 articles, three were excluded; two were excluded due to lack of data on cleft and the other was excluded because it was a review paper. Five additional studies were identified from searching the references of the 12 remaining articles. Therefore, a total of 17 articles were included in the systematic review. The flow chart showing the study selection is presented in Appendix D.

The 17 studies were published between 1946 and 2017 and were conducted across three continents. Countries involved in these studies included the United States of America, Canada, the United Kingdom, Sweden, Denmark, Hungary, Japan, and China. These articles are summarised in Table 7.

Author, Publication Date, Setting, Study Design and Study ID	Cases	Controls	Exposure	Time of Exposure	Outcome	Results Related to Exposure of Concern
Crider *et al.*, 2009 United States of America Case control study ID: 1	Mothers who had infants with at least 1 major birth defects (n = 13,155)	Mothers who had normal infants, selected from the same geographical areas (n = 4,941)	Antibiotics including **erythromycins**	**Periconceptional**	Birth defects including **OC, CL/P, CL** and **CP**	OC (n = 1,951) Exposed (n = 27) AOR 0.8 (95% CI 0.5-1.3) CL/P (n = 1269) Exposed (n = 13) AOR 0.6 (95% CI 0.3-1.1) CL (n = 431) Exposed (n = 5) AOR 0.5 (95% CI 0.2-1.5) CLP (n = 838) Exposed (n = 8) AOR 0.6 (95% CI 0.3-1.3) CP (n = 682) Exposed (n = 14) AOR 1.1 (95% CI 0.6-2.1)
Källen *et al.*, 2005 Sweden Case control study ID: 2	Infants born to mothers exposed to erythromycin	Infants born to mothers exposed to penicillin V	**Erythromycin**	Between weeks 10–12 and before the end of the 1st trimester (**periconceptional**)	MCMs including median **CP** and minor	CP (n = 2)

	(n = 3,675)	(n = 9,110)			congenital malformations	
Mølgaard-Nielsen and Hviid, 2012 Denmark Cohort study ID: 3	Mothers exposed to antibiotics (n = 98,852)	Mothers not exposed to antibiotics (n = 707,159)	Antibiotics including **erythromycin**, **roxithromycin**, and **azithromycin**	1st trimester, 2nd month and 3rd month (**periconceptional**)	**CL/P** **CP**	CL/P *Exposed:* Crude POR 1.10 (95% CI 0.91-1.33) Adjusted POR 1.08 (95% CI 0.89-1.30) *Unexposed:* Crude POR 1 (ref) Adjusted POR 1 (ref) CP *Exposed:* Crude POR 1.14 (95% CI 0.86-1.52) Adjusted POR 1.14 (95% CI 0.86-1.51) *Unexposed:* Crude POR 1 (ref) Adjusted POR 1 (ref) Erythromycin 1st trimester – CL/P (n = 14) CP (n = 4) 2nd month – CL/P (n = 3) 3rd month – CP (n = 0)

						Roxithromycin 1st trimester – CL/P (n = 3) CP (n = 1) 2nd month – CL/P (n = 0) 3rd month – CP (n = 0) Azithromycin 1st trimester – CL/P (n = 6) CP (n = 1) 2nd month – CL/P (n = 1) 3rd month – CP (n = 0)
Muanda *et al.*, 2017 Canada Cohort study ID: 4	Mothers exposed to antibiotics (n = 15,469)	Mothers not exposed to antibiotics (n = 124,469)	Antibiotics including **macrolides**, **azithromycin**, **clarithromycin**, and **erythromycin**, antiprotozoals, and urinary anti-infectives	1st trimester (**periconceptional**)	MCMs including **CP**	Macrolides (n = 2,332) CP (n = 7) (0.30%) OR 1.7 (95% CI 0.80-3.60) Azithromycin (n = 883) CP (n = 2) (0.23%) OR 1.27 (95% CI 0.32-5.08) Clarithromycin (n = 658) CP (n = 2) (0.30%) OR 1.57 (95% CI 0.39-6.41) Erythromycin (n = 697) CP (n = 3) (0.43%) OR 2.49 (95% CI 0.80-7.74)

Czeizel *et al.*, 1999 Hungary Case control study ID: 5	Pregnant women who had newborns or foetuses with CAs (n = 22,865) Exposed: (n = 113)	Pregnant women who had newborn infants without any CAs (n = 38,151) Exposed: (n = 172)	**Erythromycin**	1st month (**periconceptional**) 2nd and 3rd month of gestation (**periconceptional**) 4th-9th months (**entire pregnancy)** **Entire pregnancy**	Isolated and multiple CAs including **CL/P** and **CP**	*1st month exposure*: CL/P (n = 1) OR 1.8 (95% CI 0.2-13.2) CP (n = 1) OR 4.1 (95% CI 0.5-30.7) *2nd-3rd months exposure:* CL/P (n = 0) CP (n = 1) OR 1.5 (95% CI 0.2-10.6) *4th-9th months exposure:* CL/P (n = 3) OR 0.7 (95% CI 0.2-2.3) CP (n = 1) OR 0.6 (95% 0.1-4.1) *Entire pregnancy:* CL/P (n = 4) OR 0.6 (95% CI 0.2-1.7) CP (n = 3) OR 1.1 (95% CI 0.4-3.5) <u>McNemar Analysis</u> *Entire pregnancy exposure:* CL/P (n = 0) OR 0.8 (95% CI 0.2-3.4) CP (n = 0) OR 0.7 (95% CI 0.1-4.3)*2nd-3rd months exposure:*

						CP (n = 0) OR 0.9 (95% CI 0.1-14.7)
Czeizel *et al.*, 2003 Hungary Case control study ID: 6	Newborn infants or terminated pregnancies with CAs (n = 22,843) Exposed: (n = 68)	Paired newborn infants without any CAs (n = 38,151) Exposed: (n = 122)	Econazole (**antifungal**)	1st month (**periconceptional**) 2nd and 3rd months (**periconceptional**) 4th-9th months (**entire pregnancy)** **Entire pregnancy**	CAs including **CL/P**	*1st month exposure*: CL/P (n = 0) *2nd-3rd months exposure:* CL/P (n = 1) POR 1.3 (95% CI 0.3-10.2) *4th-9th months exposure:* CL/P (n = 1) POR 0.2 (95% CI 0.0-1.4) *Entire pregnancy exposure:* CL/P (n = 2) POR 0.3 (95% CI 0.1-1.3) <u>McNemar Analysis</u> *2nd-3rd months exposure:* CL/P (n = 0) POR 1.3 (95% CI 0.1-73.7) *Entire pregnancy exposure:* CL/P (n = 0) POR 0.1 (95% CI 0.0-0.7)
Howley *et al.*, 2016	Mothers who had infants with 1 or more	Mothers who had infants	Fluconazole (**antifungal**)	1st trimester (**periconceptional**)	Major structural birth defects	CP (n = 1) CL (n = 1)

United States of America Case control study ID: 7	major structural birth defects (n = 31,645) Exposed: (n = 44)	without birth defects (n = 11,612) Exposed: (n = 6)			including **CP**, **CL**, and **CLP**	CLP (n = 6) OR 5.53 (95% CI 91.68-18.24)
Hill *et al.*, 1988 United Kingdom Case control study ID: 8	Mothers of infants born with OC and/or major limb reduction defects (n = 791) OC: (n = 676) Limb reduction defect: (n = 115)	Mothers of infants without congenital malformations (n = 791)	Drugs including **antifungals**	3 months before last menstrual cycle (**periconceptional**) 1st trimester (**periconceptional**)	**OC** and/or major limb reduction defects	*Exposure 3 months before last menstrual cycle:* Case mothers (n = 14) Control mothers (n = 20) OC (n = 676) OR 0.7 (95% CI 0.4-1.4) *1st trimester exposure:* Case mothers (n = 17) Control mothers (n = 20) OC (n = 676) OR 0.9 (95% CI 0.4-1.6)
Jick, 1999 United Kingdom Case control study ID: 9	Pregnant women exposed to oral fluconazole during 1st trimester (n = 234)	Pregnant women exposed to topical azole during 1st trimester (n = 492)	Oral Fluconazole (**antifungal**)	1st trimester (**periconceptional**)	CAs including **CL/P**	CL/P (n = 0)

		Pregnant women not exposed to azoles during 1st trimester (n = 1,629) Pregnant women exposed to oral itraconazole during 1st trimester (n = 88)				
Mølgaard-Nielsen *et al.*, 2013 Denmark Cohort study ID: 10	Infants exposed to Fluconazole (n = 7,352)	Infants not exposed to Fluconazole (n = 968,236)	Fluconazole (**antifungal**)	1st trimester (**periconceptional**)	Major birth defects including **CL/P** and **CP**	CL/P *Exposed* (n = 10) *Unexposed* (n = 1,264) Crude POR 1.04 (95% CI 0.56-1.94) Adjusted POR 1.02 (95% CI 0.55-1.91) CP *Exposed* (n = 5) *Unexposed* (n = 497)

						Crude POR 1.33 (95% CI 0.55-3.20) Adjusted POR 1.24 (95% CI 0.51-3.00)
Carter *et al.*, 2008 United States of America Case control study ID: 11	Live births, still births (≥ 20 weeks or > 500 g), or elective terminations with birth defects and exposed to antifungals (n = 7,047)	Live births without birth defects and exposed to antifungals (n = 4,774)	**Antifungals** including Miconazole, Clotrimazole, Terconazole, Fluconazole, Tioconazole, Ketoconazole, Econazole, Butoconazole, Ciclopirox olamine, Propionic acid, and Terbinafine	1st trimester (**periconceptional**)	Isolated birth defects including **CL/P**	CL/P (n = 1,086) Miconazole (n = 9) Clotrimazole (n = 0) Terconazole (n = 2) Fluconazole (n = 2) Tioconazole (n = 1) Ketoconazole (n = 0) Econazole (n = 1) Butoconazole (n = 0) Ciclopirox olamine (n = 1) Propionic acid (n = 0) Terbinafine (n = 1) CL/P (n = 23) AOR 1.24 (95% CI 0.78-1.99)
Nørgaard *et al.*, 2008 Denmark Cohort study ID: 12	Pregnant women exposed to fluconazole during 1st trimester (n = 1,079)	Pregnant women not exposed to fluconazole during 1st trimester (n = 170,453)	Fluconazole (**antifungal**)	1st trimester (**periconceptional**)	Congenital malformations including **CL/P**	CL/P (n = 3)
Czeizel *et al.*, 1999 Hungary	Newborn infants with CAs	Newborn infants without CAs	Clotrimazole (**antifungal**)	1st month of pregnancy (**periconceptional**)	CAs including **CL/P** and **CP**	*Entire pregnancy exposure:*

Case control study ID: 13	(n = 18,515) Exposed: 7.1%	(n = 32,804) Exposed: 7.7%		2nd and 3rd months (**periconceptional**) 4th-9th months (**entire pregnancy**)		CL/P (n = 90) POR 0.93 (95% CI 0.69-1.27) CP (n = 28) POR 0.78 (95% CI 0.46-1.31) *2nd or 3rd month exposure:* CL/P (n = 18) POR 0.93 (95% CI 0.48-1.80) CP (n = 2) POR 0.52 (95% 0.09-2.86)
Cartsos *et al.*, 2012 United States of America Cohort study ID: 14	CLP infants born to mothers exposed to antiretrovirals (n = 26)		**Antiretrovirals** including Efavirenz, Lamivudine, Abacavir sulphate/ Lamivudine/ Zidovudine, Nelfinavir mesylate, Stavudine, Nevirapine, Lopinavir/ Ritonavir, and Lamivudine/ Zidovudine	**Entire pregnancy**	**CLP**	Efavirenz CLP (n = 6) ROR 196.01 (95% CI 85.89-447.32) Lamivudine CLP (n = 5) ROR 60.23 (95% CI24.53-147.89) Abacavir sulphate/ Lamivudine/ Zidovudine CLP (n = 2) Nelfinavir mesylate CLP (n = 2) ROR 50.53 Stavudine CLP (n = 1) ROR 29.57

						Nevirapine CLP (n = 3) ROR 27.59 (95% CI 8.75-86.99) Lopinavir/ Ritonavir CLP (n= 5) ROR 26.47 (95% CI 10.78-64.97) Lamivudine/ Zidovudine CLP (n = 3) ROR 24.94 (95% CI 7.91-78.62)
Minakami *et al.*, 2014 Japan Retrospective review ID: 15	Pregnant women exposed to Laninamivir (n = 112)		Laninamivir (**antiretroviral**)	≤ 21 weeks (**entire pregnancy**) 22-36 weeks (**entire pregnancy**) ≥ 37 weeks (**entire pregnancy**)	Adverse pregnancy outcomes including **CL**	CL (n = 1)
Liu *et al.*, 2013 China Cohort study ID: 16	Pregnant women exposed to Telbivudine (n = 86)		Telbivudine (**antiviral**)	**Entire pregnancy**	Adverse pregnancy outcomes including **CLP**	CLP (n = 1)
Townsend *et al.*, 2009 United Kingdom and Ireland Cohort study	Infants with CAs born to mothers diagnosed with		**Antiretrovirals** including nucleoside reverse transcriptase	Early – 1st trimester (**periconceptional**)	Major and minor CAs including **CL/P**	*Early exposure:* CLP (n = 1) *Late exposure:* CLP (*n = 5)*

ID: 17	HIV before delivery (n = 232) Unexposed: (n = 14) Exposed during 1st trimester: (n = 53) Exposed during 2nd and 3rd trimester: (n = 147)		inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors, or both NNRTIs and protease inhibitors	Late – 2nd and 3rd trimester (**entire pregnancy**)		*Unexposed:* CLP (n = 1) Total: CL/P (n = 7)

Table 7 Summary of included studies.

4.1.1 Macrolides

Antibiotics are one of the most commonly used medications during pregnancy. They are indicated to treat infections, which is crucial for the health and wellbeing of both the mother and the foetus. Macrolides are a class of antibiotics that inhibit protein synthesis in bacteria, affecting gram-positive cocci and intracellular pathogens, such as mycoplasma and chlamydia. They are mainly bacteriostatic, but at higher concentrations they may be bactericidal to some types of microorganisms.

Macrolides accounted for five of the 17 studies included in this systematic review. Erythromycin, the most widely known macrolide and first to be discovered, was investigated in all five studies. Other macrolides, such as azithromycin, clarithromycin, and roxithromycin, were also assessed in two of these studies. All five observational studies concluded that erythromycin is not associated with an increased risk of OFC.

4.1.1.1 Muanda *et al.*, 2017

This population-based cohort study aimed at investigating the association between specific antibiotics and MCMs. Using the Quebec pregnancy cohort, all pregnant women covered by the Quebec Public Prescription Drug Insurance from January 1998 to December 2009 were included in the study. Out of the 139,938 pregnancies included, 15,469 were exposed to antibiotics and 124,469 were not exposed. Macrolides were the second most frequently used antibiotics, accounting for 15% of the pregnancies. Confounding factors, such as sociodemographic variables, maternal chronic co-morbidities, endometriosis and maternal infections, measures of healthcare one year prior to pregnancy, year of delivery, and infant gender, were all accounted for. This population-based cohort study concluded that *in utero*

exposure to macrolides was associated with an increased risk of organ-specific MCMs in infants, specifically the digestive system, with a 46% increased risk. Erythromycin was associated with an increased risk of urinary system malformations. However, as stated by the authors, this could have been a result of chance or due to residual confounding. Macrolides were not associated with an increased risk of CP.

4.1.1.2 Mølgaard-Nielsen and Hviid, 2011

The aim of this nationwide cohort study was to investigate the risk of OFC among pregnant women exposed to antibiotics. The study included a total of 806,011 single live births in Denmark from January 1, 1996 to September 30, 2008, from which 945 had CL/P and 400 had isolated CP. From the total, only 98,852 were exposed to antibiotics. Potential confounders were adjusted for including birth year, maternal sociodemographic variables, socioeconomic status, maternal smoking, maternal co-morbidities, and use of other maternal drugs. Mølgaard-Nielsen and Hviid concluded that maternal exposure to antibiotics, including erythromycin, roxithromycin, and azithromycin, early in pregnancy was not associated with an increased risk of CL/P or CP.

4.1.1.3 Crider *et al.*, 2009

The National Birth Defects Prevention Study is an ongoing population-based case control study of birth defects across ten states in the United States of America. The aim of this case control study was to investigate the association between antibiotics taken during pregnancy and selected birth defects. The study comprised 13,155 case mothers who had an infant with at least one major birth defect and 4,941 control mothers from the same geographical region. The findings were reassuring in that erythromycin, which was the second most

widely reported antibacterial used during pregnancy in that population group, was not associated with many adverse birth defects, including OC. Only anencephaly and transverse limb deficiency were seen to be associated with periconceptional use of erythromycin.

4.1.1.4 Källen *et al.*, 2005

This case control study aimed to study the effects of maternal erythromycin intake during early pregnancy. From the Swedish Medical Birth Registry, 677,028 infants born between July 1, 1995 and December 31, 2002, whose mothers were exposed to erythromycin, were included in the study. These cases were compared to 9,110 infants who were exposed to penicillin V during early pregnancy. Confounding factors taken into consideration included infant's year of birth, maternal age, parity, maternal smoking, earlier miscarriages, and use of other drugs. Unlike the other studies, this study indicated a teratogenicity of erythromycin when taken early in pregnancy, causing an increased risk of developing any congenital malformation. The authors have suggested that this may be due to the adverse effects on the cardiovascular system. Erythromycin has been seen to inhibit a specific cardiac potassium current channel, which in turn affects the cardiac rhythm in embryos. In addition to cardiovascular malformations, the study also shows an association between erythromycin and pyloric stenosis. Unfortunately, the authors only briefly mention cleft in a table. Out of the 62 cases that presented with MCMs after exposure to erythromycin in early pregnancy, only two cases had CP.

4.1.1.5 Czeizel *et al.*, 1999

The third population-based case control study included in this systematic review also aimed at studying the teratological effects of erythromycin. Cases and controls were derived from

the Hungarian Case-Control Surveillance of Congenital Abnormalities between 1980 and 1996. The study consisted of 22,865 infants born with CAs, of which 113 were exposed to erythromycin, and 38,151 infants born without CAs, of which 172 were exposed to erythromycin. Mothers who were on other pregnancy supplements, such as vitamins, iron, and calcium derivatives, were excluded. The study showed no increased risk of CAs, including CL/P and CP, in babies exposed to erythromycin during the second and third months of pregnancy, which is a critical time during which CAs can develop in a foetus.

4.1.2 Antifungals

Antifungals, also known as antimycotics, are fungicidal or fungistatic drugs used to treat fungal infections. There are two types of antifungals: local (topical or vaginal) or systemic (oral or intravenous) and are classified as polyenes, azoles, allylamines, echinocandins, and miscellaneous antifungals. Some of the most common antifungals include clotrimazole, econazole, miconazole, terbinafine, fluconazole, ketoconazole, and amphotericin.

Pregnant women are at great risk of developing vulvovaginal candidiasis, and topical azoles are the first line of treatment for these cases. The Food and Drug Administration (FDA) guidelines and the Centres for Disease Control and Prevention (CDC) recommendations suggest avoiding oral fluconazole for the treatment of vulvovaginal candidiasis in pregnancy. Despite these guidelines, fluconazole is still being prescribed today (Howley *et al.*, 2016).

This systematic review includes seven studies investigating antifungal medications, five of which provide data on *in utero* fluconazole exposure. Four of these studies concluded that there is no association between fluconazole and the development of CL/P. However, the

most current article states that there is a significant association between fluconazole use and CLP.

4.1.2.1 Howley *et al.*, 2016

As fluconazole is commonly prescribed to treat vulvovaginal candidiasis, Howley *et al.* (2016) investigated the potential teratogenicity of fluconazole during the first trimester of pregnancy. This population-based case control study included all pregnancies with delivery dates between October 1, 1997 and December 31, 2011. Cases comprised 31,645 mothers who had infants with at least one major structural birth defect and 11,612 mothers who had infants without any birth defects. Of the 43,257 mothers analysed, 44 case mothers and six control mothers were exposed to fluconazole during the first trimester. This study showed a significant association between fluconazole and CLP (n = 6) and d-transposition of the great arteries (n = 3). This supports the CDC recommendations and the FDA guidelines, which suggest avoiding fluconazole for the treatment of vulvovaginal candidiasis during pregnancy. However, the findings should be interpreted with caution as the sample size for exposed cases was small.

4.1.2.2 Mølgaard-Nielsen *et al.*, 2013

This population-based cohort study investigated the association between first trimester oral fluconazole and the risk of 15 major birth defects known to be associated with azole antifungals. All 976,300 live births from January 1, 1996 to March 31, 2011 from the Medical Birth Registry in Denmark were included in the study. Of these, 7,352 infants were exposed to fluconazole and 967,236 were not exposed. Reassuringly, the authors found no increased risk of 14 of the 15 birth defects, including CL/P and CP. Tetralogy of Fallot was the only

birth defect that was significantly associated with fluconazole exposure during early pregnancy.

4.1.2.3 Carter *et al.*, 2008

The aim of this case-control study was to use data from the National Birth Defects Prevention Study from 1997 to 2003 to investigate the association between first trimester antifungal use and the risk of selected birth defects. The antifungal drugs included in this study are listed in Table 7, of which miconazole was the most commonly used. Miconazole, a topical azole, was mainly used to treat vulvovaginal candidiasis, except in three cases where it was used to treat ringworm, a fungal skin infection, and oral thrush. The cases totalled 7,047 babies, which included live births, still births (≥ 20 weeks or > 500 g), or elective terminations with birth defects. Controls, on the other hand, were 4,774 live births without any birth defects. Both cases and controls were exposed to antifungals during the first trimester. This study found no association with first trimester exposure to antifungals and most birth defects including CL/P. However, there was an increased risk for hypoplastic left heart syndrome.

4.1.2.4 Nørgaard *et al.*, 2008

This was the third of four studies included in this systematic review that purely focused on examining fluconazole. Although fluconazole is commonly used to treat candidiasis, there is limited data available on its potential side effects. This population-based cohort study aimed to examine any association between first trimester fluconazole and the risks of congenital malformations. From the Danish Medical Registry, 171,532 mothers who had a live birth or stillbirth from January 1, 1973 were selected. Of these, 1,079 were case mothers exposed to

fluconazole during the first trimester, and there were 170,453 controls. Case mothers gave birth to 44 (4.1%) infants with congenital malformations and control mothers gave birth to 6,152 (3.6%) infants with congenital malformations. The authors concluded that there is no increased risk of congenital malformations, one of which was CL/P, when exposed to fluconazole during the first trimester of pregnancy.

4.1.2.5 Czeizel *et al.*, 2003

Czeizel *et al.* (1999) had earlier conducted a case control study investigating clotrimazole. Four years later, they conducted a similar study, this time on econazole, another topical azole antifungal. The aim was to assess the risks and benefits of vaginal econazole treatment during pregnancy. Cases, which included either live births or terminated pregnancies with isolated or multiple CAs, were selected from the Hungarian Congenital Abnormality Registry. Each case was matched according to sociodemographic variables with two live birth controls without any CAs. Of the 22,843 cases, 68 infants were exposed to econazole, and of the 38,151 controls, 122 were exposed to econazole. The matched case-control pairs did not show evidence of teratogenicity associated with vaginal econazole when taken in low doses, including CL/P.

4.1.2.6 Jick, 1999

In this case control study, which aimed at evaluating the risks of maternal exposure to fluconazole during the first trimester, 234 exposed women were selected from the General Practice Research Database in the United Kingdom. These cases were matched with three control groups:

1. 492 women exposed to topical azoles, including miconazole, ketoconazole, and econazole
2. 88 women exposed to oral itraconazole
3. 1,629 women not exposed to azoles during the first trimester of pregnancy

Out of these four groups, there were only two cases of CL/P, one exposed to topical azole and the other not exposed to any azole. The authors concluded that there is no association between CAs and infants exposed to fluconazole during the first trimester.

4.1.2.7 Czeizel *et al.*, 1999

This case control study was conducted to examine the teratogenic effects of clotrimazole during pregnancy. Infants with isolated and multiple CAs born between 1980 and 1992 were selected from the Hungarian Case-Control Surveillance of Congenital Abnormalities. The study included 18,515 cases and 32,804 controls of which 7.1% and 7.7% were exposed to clotrimazole, respectively. Potential confounding factors, such as maternal age, birth order, pregnancy complications, acute and chronic maternal disorders and use of other medications, were taken into consideration. The data presented did not show any association between clotrimazole exposure during pregnancy and multiple CAs, including the risk for CL/P and CP. However, there was evidence for a reduction in the prevalence of undescended testis.

4.1.2.8 Hill *et al.*, 1988

Around 1988, evidence was available linking limb reduction defects to periconceptional intake of thalidomide and OC to anticonvulsants. However, there was limited evidence on the effects of other drugs on these two CAs. Therefore, this study was undertaken in the

United Kingdom to assess the effects of periconceptional drugs on limb reduction defects and OC. From the Office of Population Censuses and Surveys, all infants born with limb reduction defects and/or OC within one year from October 1, 1983 were included in the study. One hundred and fifteen mothers delivered infants with limb reduction defects and 676 mothers had infants with OC. These cases were matched with an equal number of mothers who had the next normal infant in the same general practice. Like previous studies, this study confirmed that anticonvulsant drugs are significantly associated with an increased prevalence of OC. There was also a significant association between oral contraceptives and cleft development. However, there was no increased risk with antifungal exposure. Regarding limb reduction defects, there was no significant association with the listed exposures, and the authors disregarded this part of the study due to the small sample size in comparison to the OC cases.

4.1.3 Antivirals/ Antiretrovirals

Antiviral drugs are a class of antimicrobials used to treat viral infections. Most antivirals available on the market are designed to target the human immunodeficiency viruses (HIV), herpes viruses, hepatitis B and C viruses, and influenza A and B viruses. Unlike most antibiotics, antivirals do not destroy the pathogen, but rather they either inhibit their development before entering the cell or interfere with the processes that synthesize viral components after they have entered the cell. The latter approach is undertaken by specific antivirals known as antiretrovirals. Antiretrovirals are used to treat HIV and are usually used in combination. They include nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and protease inhibitors. Research suggests that the use of antivirals and antiretrovirals during pregnancy is crucial to prevent further complications

and to prevent mother-to-child transmission of the virus. Therefore, the benefits outweigh the potential teratogenic risks.

This systematic review included two studies on antivirals and two on antiretrovirals. However, because these four studies are heterogenous in the type of exposure, time of exposure, and cleft phenotype, an overall conclusion cannot be made. There was also insufficient data for this group of antimicrobials to be included in the meta-analysis.

4.1.3.1 Minakami *et al.*, 2014

The Centers for Disease Control and Prevention (2019) states “Influenza (flu) is more likely to cause severe illness in pregnant women than in women of reproductive age who are not pregnant” (CDC, 2019). Therefore, it is crucial to treat infected, pregnant women with antivirals. However, with the development of resistance against non-teratogenic antivirals, the teratogenicity of other antivirals must be studied. This retrospective study aimed to evaluate pregnancy outcomes when exposed to laninamivir, a new antiviral medication on the market. Fifty members of the Japan Society of Obstetrics and Gynaecology and Japan Association of Obstetricians and Gynaecologists were asked to provide data on pregnant women who were prescribed laninamivir for the treatment of influenza during two time periods:

1. October 1, 2011 to March 31, 2012
2. October 1, 2012 to March 31, 2013

Half of the physicians prescribed laninamivir to 112 pregnant women. Exposure was reported in three categories: $\leq$ 21 weeks, 22-36 weeks, and $\geq$ 37 weeks of gestation, and adverse events, such as miscarriages, preterm birth, congenital malformations, neonatal

and morbidity requiring treatment were evaluated. At ≤ 21 weeks of exposure, one mother had a miscarriage, nine had preterm births, and three had congenital malformations (CL, foot polydactyly, and farefoot varus deformity). Although there were no controls, this study suggests that there is no increased risk of adverse events when taking laninamivir during pregnancy.

4.1.3.2 Liu *et al.*, 2013

This was the second paper included in this systematic review investigating antivirals. Telbivudine is the first line of treatment for chronic hepatitis B (CHB) during pregnancy. Recently, telbivudine has been administered to pregnant women during the third trimester to prevent mother-to-infant transmission of the virus. Although telbivudine is considered safe, there is limited information on the safety of telbivudine for the "entire" pregnancy. This cohort study aimed to assess this period of exposure. Between October 1, 2007 and May 31, 2012, all women intending to become pregnant or were < 12 weeks pregnant and had been diagnosed with CHB at the outpatient clinic in Beijing Ditan Hospital were included in the study. Eighty-six pregnant women exposed to telbivudine were followed up throughout the course of pregnancy as were their infants post-delivery. At the end of the follow-up period, 50 mothers delivered 52 full-term infants and 31 mothers were still pregnant. One mother had induced labour at 24 weeks of gestation to terminate her pregnancy for CLP. The other CA recorded was right ear accessories, which occurred in one infant. With an overall CA rate of 3.8%, the authors confirmed the safety of telbivudine in the treatment of CHB-infected pregnant mothers for the entire pregnancy.

4.1.3.3 Cartsos *et al.*, 2012

Antiretroviral drugs are also prescribed during pregnancy to prevent mother-to-child transmission of HIV. This cohort study specifically assessed the risk of CLP when pregnant women were exposed to antiretroviral prophylaxis. The FDA Adverse Events Reporting system database was searched, and a total of 26 CLP cases related to antiretroviral therapies were identified. The therapies were either monotherapies or combination therapies. This study was the first to find an association between antiretroviral drugs and the development of CLP. Listed below are the drugs from greatest (at the top) to least risk of an infant developing a cleft:

- Efavirenz
- Lamivudine
- Abacavir sulphate/lamivudine/zidovudine
- Nelfinavir
- Nevirapine
- Lopinavir/ritonavir
- Lamivudine/zidovudine

4.1.3.4 Townsend *et al.*, 2009

This cohort study was the second antiretroviral study included in this systematic review. The aim of the study was to assess the association between *in utero* exposure to antiretroviral drugs and the development of CAs. From the National Study of HIV in Pregnancy and Childhood, 8,576 infants (live births or stillborn), born between 1990 and 2007 in the United Kingdom and Ireland, were included. Of the 8,242 infants that had information on CAs, only

232 infants had at least one CA. This resulted in an overall CA rate of 2.8%. Fourteen infants were not exposed to antiretrovirals, 53 were exposed following the first trimester, and 147 were exposed following the second or third trimester. Regarding OFC, there were only seven infants with CL/P; five of which were exposed to antiretrovirals during the second or third trimester, one during the first trimester, and the other was not exposed at all. This study found no association with CAs and the type of antiretroviral exposure. Nor did they find an association with time of exposure. The authors concluded that *in utero* exposure to antiretrovirals is not associated with an increased risk to CAs.

4.2 Variables Included in the Meta-Analysis

Across the 17 studies included in this systematic review, there was a wide range of heterogeneity in terms of exposure, time of exposure, and outcome. In order to conduct a meta-analysis, these factors had to be grouped in such a way that the articles could be related quantitatively.

4.2.1 Exposure

This systematic review categorised the exposure into the following three groups:

- Macrolides
- Antifungals
- Antivirals/ antiretrovirals

However, for the meta-analysis, it was decided to exclude antivirals/ antiretrovirals as there was insufficient homogenous data available. The different subtypes of antimicrobial categories were also disregarded in the meta-analysis as their inclusion would in turn increase the heterogeneity in the analysis.

4.2.2 Time of Exposure

Different *in utero* exposure times were assessed across the 17 papers, and they have been divided into two categories:

- Periconceptional period of pregnancy
- Entire pregnancy

Periconceptional is defined as the period before conception to the end of the first trimester. Therefore, any study that mentioned “periconception*”, “first trimester”, “first month”, “second month”, or “third month” were grouped under the periconceptional period of pregnancy. The second category included studies that assessed the “fourth through ninth months” and “entire pregnancy”.

4.2.3 Outcome

This systematic review addressed NSOFC. The studies investigated the effects of antimicrobials on different cleft phenotypes, which included CL, CP, CL/P, and CLP. To simplify the meta-analysis, the outcome was divided into two groups:

- CL/P
- CP

The CL/P group included CL, CLP, and CL/P. The CP group comprised CP alone. Data on OC as a broad term was available in three papers (Crider *et al.*, 2009, Hill, 1988, and Mølgaard-Nielsen *et al.*, 2013). It was decided to excluded OC from the meta-analysis as it was not possible to categorise OC under either CL/P or CP.

4.3 Critical Appraisal

As previously mentioned, the studies included in this review were individually appraised using the online CASP checklist. The quality assessment checklists for the case control and cohort studies are presented in Tables 8 and 9, respectively. Irrespective of the study design, the headings, “A”, “B”, and “C”, in the second row correspond to three questions:

A. Are the results of the study valid?
B. What are the results?
C. Will the results help locally?

The numbers in the third row are questions that help answer “A”, “B”, and “C”. The first column with a designated study number corresponds to the study IDs from Table 7. A “✓” shows that this part of the appraisal was done appropriately, whereas a “X” means it was not done appropriately. If the answer to a question is unknown, a “?” is assigned.

Case Control Studies												
	A							B			C	
	1	2	3	4	5	6a	6b	7	8	9	10	11
Study 1	✓	✓	✓	✓	X	✓	✓	*	*	✓	✓	✓
Study 2	✓	✓	✓	?	✓	✓	✓	*	*	✓	✓	X
Study 5	✓	✓	✓	✓	✓	✓	✓	*	*	✓	✓	✓
Study 6	✓	✓	✓	✓	✓	✓	✓	*	*	✓	✓	✓
Study 7	✓	✓	✓	✓	X	✓	✓	*	*	✓	✓	X
Study 8	✓	✓	✓	X	✓	✓	X	*	*	X	✓	✓
Study 9	✓	✓	✓	✓	✓	?	X	*	*	X	✓	X
Study 11	✓	✓	✓	✓	X	✓	✓	*	*	✓	✓	✓
Study 13	✓	✓	✓	✓	X	✓	✓	*	*	✓	✓	✓

Table 8 CASP of case control studies included in the review.

✓ Yes
X No
? Unknown
* See Table 7

A: Are the results of the study valid?
1 – Did the study address a clearly focused issue?
2 – Did the authors use an appropriate method to answer their question?
3 – Were the cases recruited in an acceptable way?
4 – Were the controls selected in an acceptable way?
5 – Was the exposure measured to minimise bias?
6a – Aside from the experimental intervention, were the groups treated equally?
6b – Have the authors taken account the potential confounding factors in the design and/or in their analysis?

B: What are the results?
7 – How large was the treatment effect?
8 – How precise was the estimate of the treatment effect?
9 – Do you believe the results?

C: Will the results help locally?
10 – Can the results be applied to the local population?
11 – Do the results of the study fit with other available evidence?

Cohort Studies														
	A								B			C		
	1	2	3	4	5a	5b	6a	6b	7	8	9	10	11	12
Study 3	✓	✓	✓	✓	✓	✓	?	✓	*	*	✓	✓	X	✓
Study 4	✓	✓	✓	✓	X	✓	✓	✓	*	*	✓	✓	✓	✓
Study 10	✓	✓	✓	✓	X	✓	✓	✓	*	*	✓	✓	✓	✓
Study 12	✓	✓	✓	✓	X	✓	?	✓	*	*	✓	✓	✓	✓
Study 14	✓	✓	✓	✓	X	X	?	?	*	*	X	X	X	X
Study 15	✓	X	X	✓	X	X	✓	✓	*	*	✓	✓	?	✓
Study 16	✓	✓	✓	✓	X	X	✓	✓	*	*	✓	✓	✓	✓
Study 17	✓	✓	✓	X	X	✓	✓	?	*	*	X	✓	✓	✓

Table 9 CASP of cohort studies included in the review.

✓ Yes
X No
? Unknown
* See Table 7

A: Are the results of the study valid?
- 1 – Did the study address a clearly focused issue?
- 2 – Was the cohort recruited in an acceptable way?
- 3 – Was the exposure accurately measured to minimise bias?
- 4 – Was the outcome accurately measured to minimise bias?
- 5a – Have the authors identified all important confounding factors?
- 5b – Have they taken account of the confounding factors in the design and/or analysis
- 6a – Was the follow up of subjects complete enough?
- 6b – Was the follow up of subjects long enough?

B: What are the results?
- 7 – What are the results of this study?
- 8 – How precise are the results?
- 9 – Do you believe the results?

C: Will the results help locally?
- 10 – Can the results be applied to the local population?
- 11 – Do the results of this study fit with other available evidence?
- 12 – What are the implications of this study for practice?

4.4 Risk of Bias

Figure 5 illustrates the risk of bias associated with each study included in the systematic review, as well as the overall risk of bias. Figure 6 shows the weighted bar plot of the

distribution of risk of bias judgements within each domain. Eight articles in the analysis were judged to have low to medium risk of bias and nine were judged to have serious risk. A major criticism of this tool is that it is completely subjective and even if one domain is judged as serious, the "overall" risk of bias is said to be at serious risk (Sterne *et al.*, 2016). Therefore, these figures must be interpreted with caution.

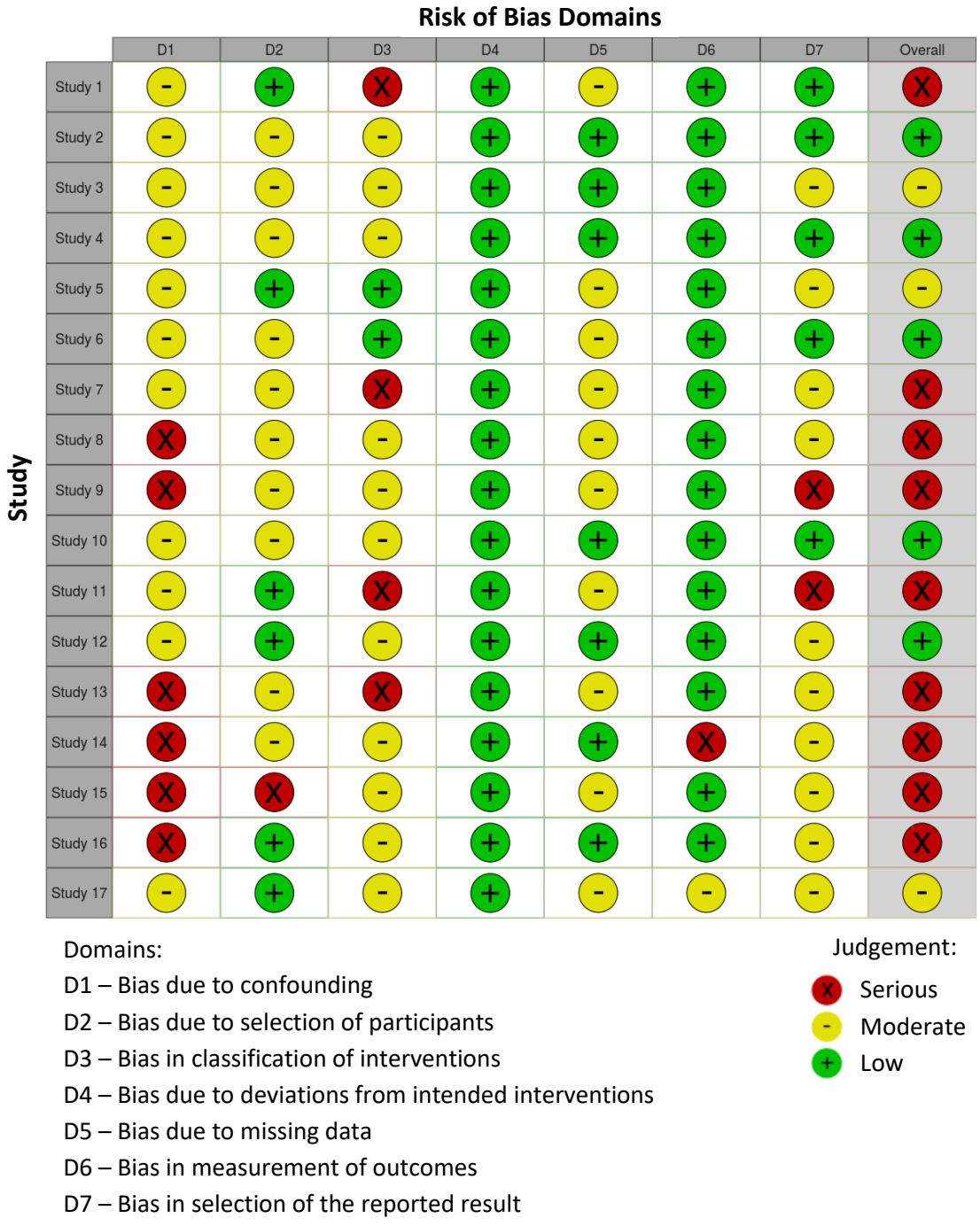

Figure 5 Traffic light plot.

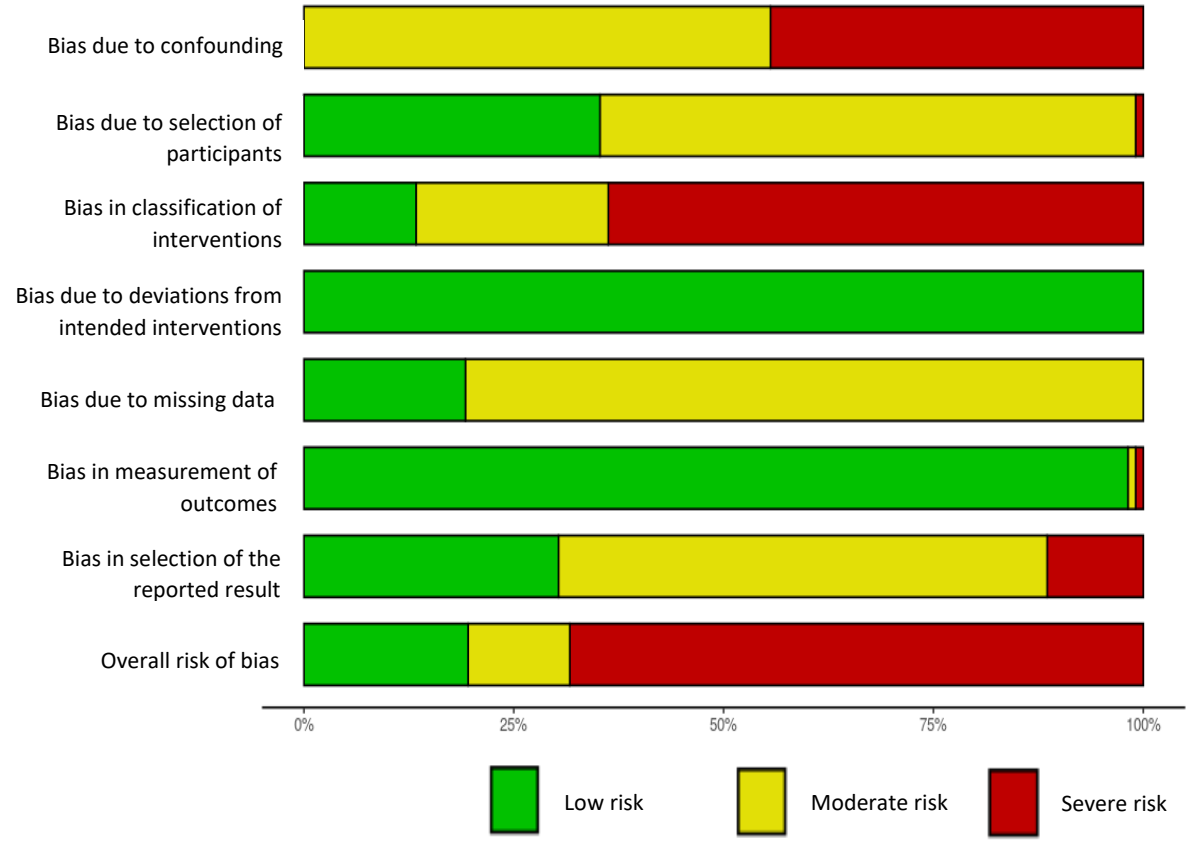

Figure 6 Weighted bar plot.

4.5 Results of the Meta-Analysis

Using the inclusion and exclusion criteria, 17 studies were included in the systematic review. Of the 17 studies, only 13 were initially included in the meta-analysis. The four articles excluded were those pertaining to antivirals/ antiretrovirals (Minakami *et al.*, 2014; Liu *et al.*, 2013; Cartsos *et al.*, 2012; Townsend *et al.*, 2009). However, the meta-analysis was limited to those measures that were either an “Odds Ratio” or “Prevalence Odds Ratio”. Therefore, we managed to extract data from eight of the 13 studies, which contained considerable clinical homogeneity (Crider *et al.*, 2009; Muanda *et al.*, 2017; Czeizel *et al.*, 1999; Czeizel *et al.*, 2003; Howley *et al.*, 2016; Mølgaard-Nielsen *et al.*, 2013; Carter *et al.*, 2008; Czeizel *et al.*, 1999).

A random effects meta-analysis was conducted to estimate the effect of macrolides and antifungals on the risk of developing a cleft. Antivrials/ antiretrovirals were not included in the meta-analysis as insufficient data could be obtained from the papers identified in the review. The data were analysed using Stata 16.1 (Stata Corp, College Station USA) statistics package. A random effects meta-analysis was used as it ensures less influence of larger studies on summary approximations. Odds Ratios and their upper and lower 95% confidence intervals (CI) where chosen as effect sizes (Hedges g) and plotted as Forest plots. The data were also analysed by subgrouping on cleft phenotype and timing of exposure during pregnancy (periconceptual and entire pregnancy). The heterogeneity between the studies was estimated using τ^2 along with I^2 and potential bias examined using contoured funnel plots with contours of statistical significance. If studies are missing in areas of low statistical significance, asymmetry may be due to publication bias, whereas when missing in areas of high statistical significance then publication bias is less likely to be the cause of the asymmetry (Palmer *et al*., 2008).

It can be seen from the Forest plot (Figure 7), which includes the result of the studies looking at both macrolides and antifungals that the summary diamond for the OR crosses the line of no effect. In addition, the Hedge's g, which is used to calculate the effect size and associated 95% CI, shows that the 95% CI includes zero (-0.1 (95% CI -0.17, 0.15)). Therefore, the analysis of the data from all the included studies would suggest that overall, there is no statistically significant effect of these two drugs on cleft development at the level of $\alpha = 0.05$.

When each drug type is considered separately, as in the Forest plot shown in Figure 9, once again in both cases it can be seen the summary diamond crosses the line of no effect and

the 95% CI for Hedge's g in both cases include zero (Macrolides: 0.30 (95% CI -0.25, 0.31) (Antifungals: -0.30 (95% CI -0.22, 0.17)), confirming neither of the drug types included appear to lead to a statistically significant increase in the likelihood of the baby developing a cleft.

When the Forest plot for individual cleft type is considered (Figure 11) it once again confirms no overall effect of drug type on cleft development with both summary diamonds crossing the line of no effect and the 95% CI of Hedge's g including zero for CL/P, CL and CLP (CL/P, CL, CLP: -0.18 (95% CI -0.51, 0.15)), and similarly for CP only (CP: -0.01 (95% CI -0.17, 0.15)).

Similarly, when the time during which either macrolides or antifungals were used, namely periconceptual or over the entire pregnancy were considered, there was once again no statistically significant effect as shown on the Forest plot in Figure 13. Both summary diamonds cross the line of no effect and the 95% CI of Hedge's g includes zero. For periconceptual it was 0.20 (95% CI -0.03, 0.42) and for the entire pregnancy it was -0.26 (95% CI -0.51, 0.00).

To perform a random effects meta-analysis an assumption is made that the data are homogenous. Measures of heterogeneity are τ^2 and I^2. If τ^2 is <0.25 then the heterogeneity is considered low or "small" and I^2 is given as a more subject percent score. In the Forest plot of all the studies combined (Figure 7) both values were 0.00, indicating the data was relatively homogenous. When the data was considered by drug it was also seen to be homogenous with the τ^2 being 0.00 as was the I^2 for antifungals, and it was still a very low 0.7% for macrolides (Figure 9). In fact, the greatest value for heterogeneity was with cleft phenotype when with CL/P, CL, and CLP the I^2 was still only 39.08%.

Overall publication bias was assessed using contour enhanced funnel plots (Figures 8, 10, 12 and 14). It can be seen in each case the results of the individual studies are symmetrically distributed around zero, suggesting publication or other potential biases were low.

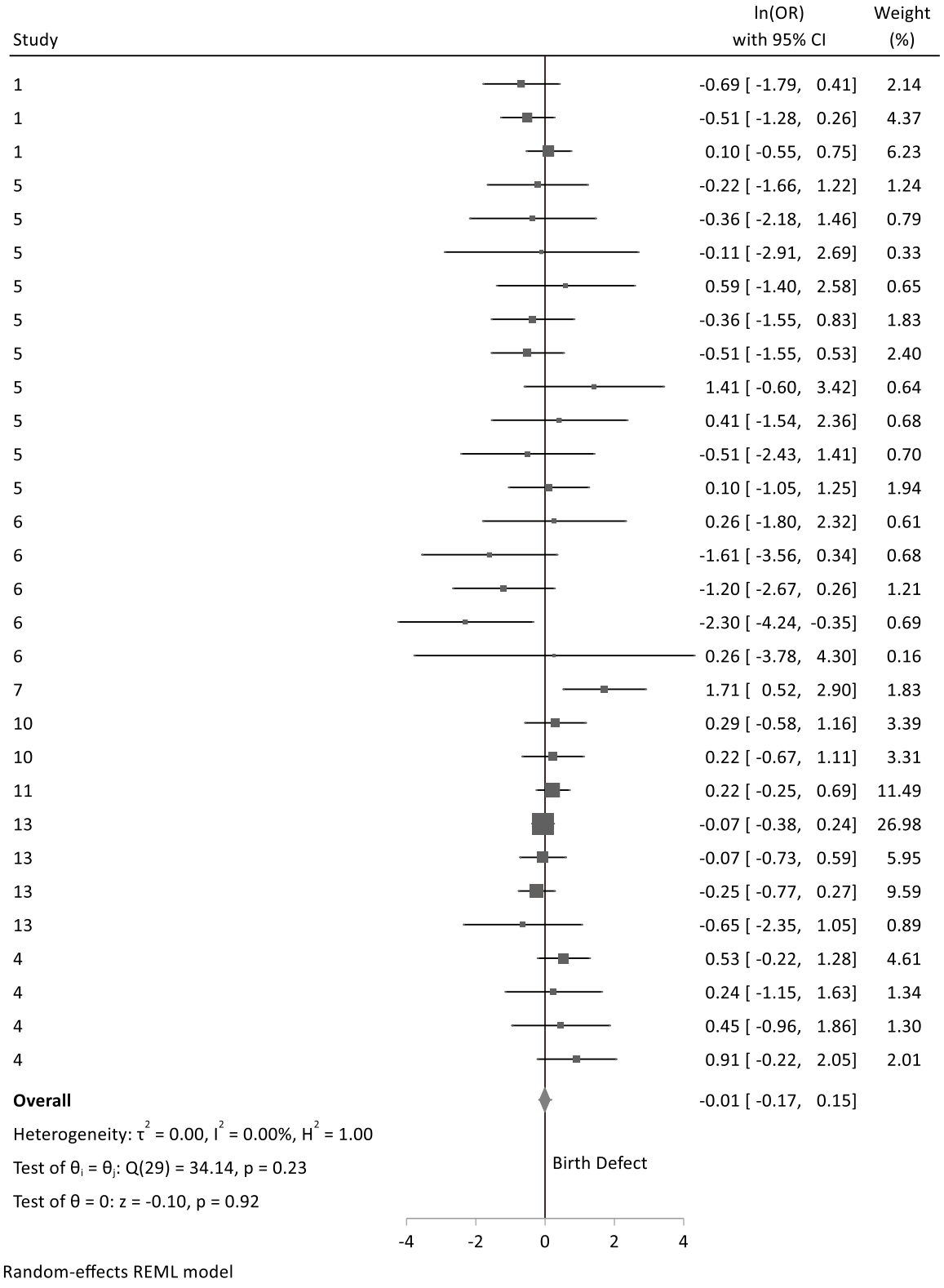

Figure 7 Forest plot illustrating the log (In) of the odds ratio (OR) and 95% confidence interval (CI) of all included studies in the meta-analysis (*i.e.* OR of 1 = chance of event or outcome = 0 on the Forest plot). Overall, the summary diamond crosses the line of no effect indicating no overall statistically significant effect of macrolides and antifungals on clefting. The study ID corresponds with the study ID values in Table 7.

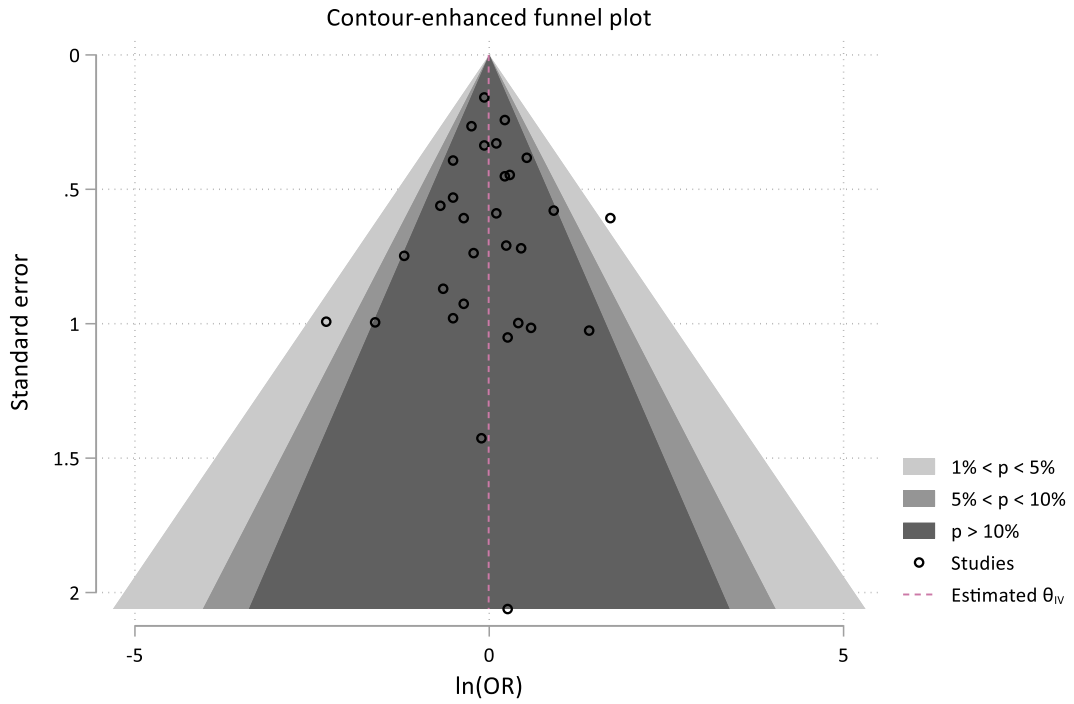

Figure 8 Funnel plot illustrating the publication bias of all the studies included in the meta-analysis.

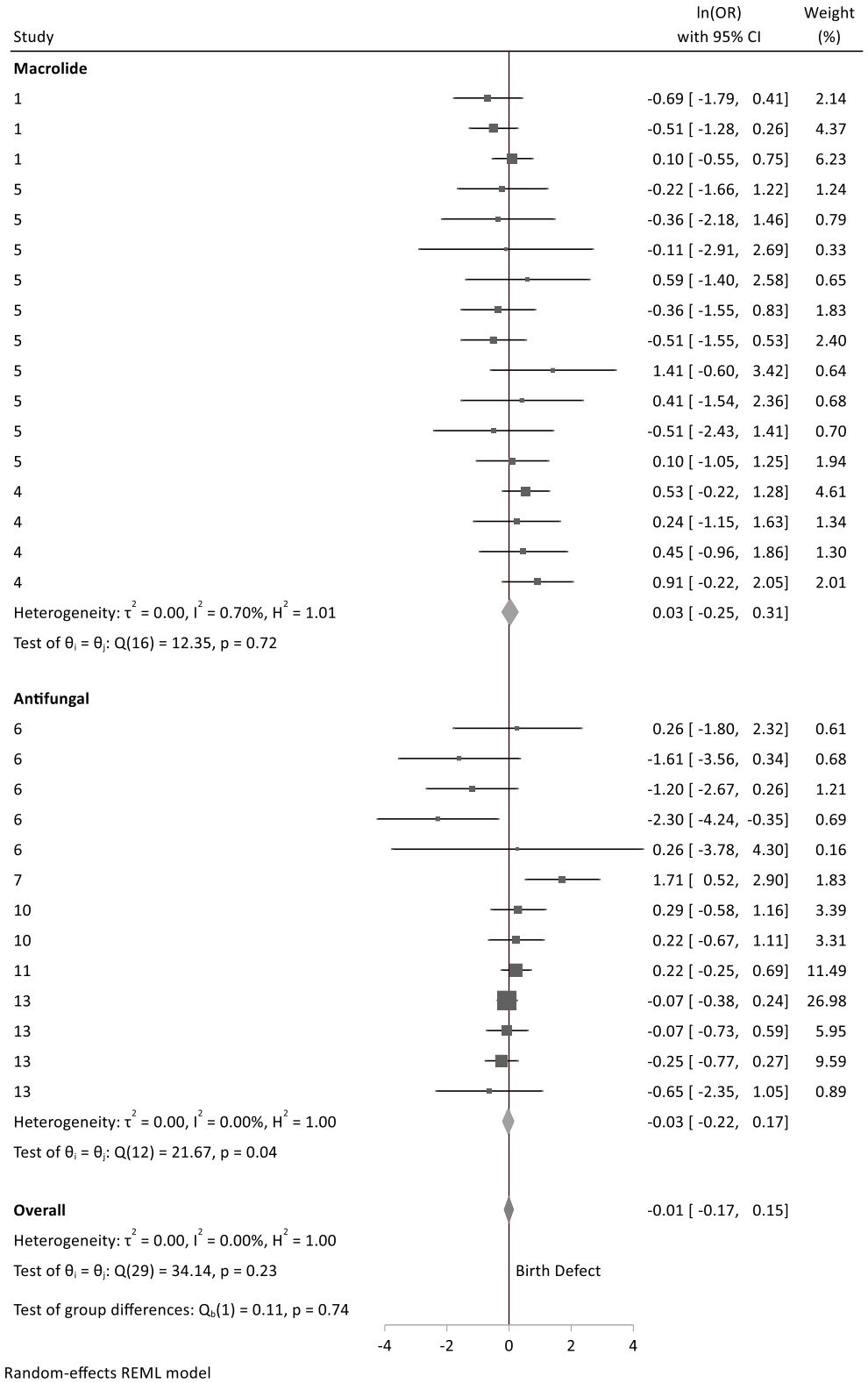

Figure 9 Forest plot of studies reporting the log (ln) of the OR and 95% CI for cleft development when exposed to macrolides and antifungals (*i.e.* OR of 1 = chance of event or outcome = 0 on the Forest plot). The upper forest plot shows the OR of cleft when exposed to macrolides *in utero* (studies 1, 4, and 5), and the lower forest plot shows the OR of cleft when exposed to antifungals *in utero* (studies 6, 7, 10, 11, and 13). In both cases the diamond crosses the line of no effect.

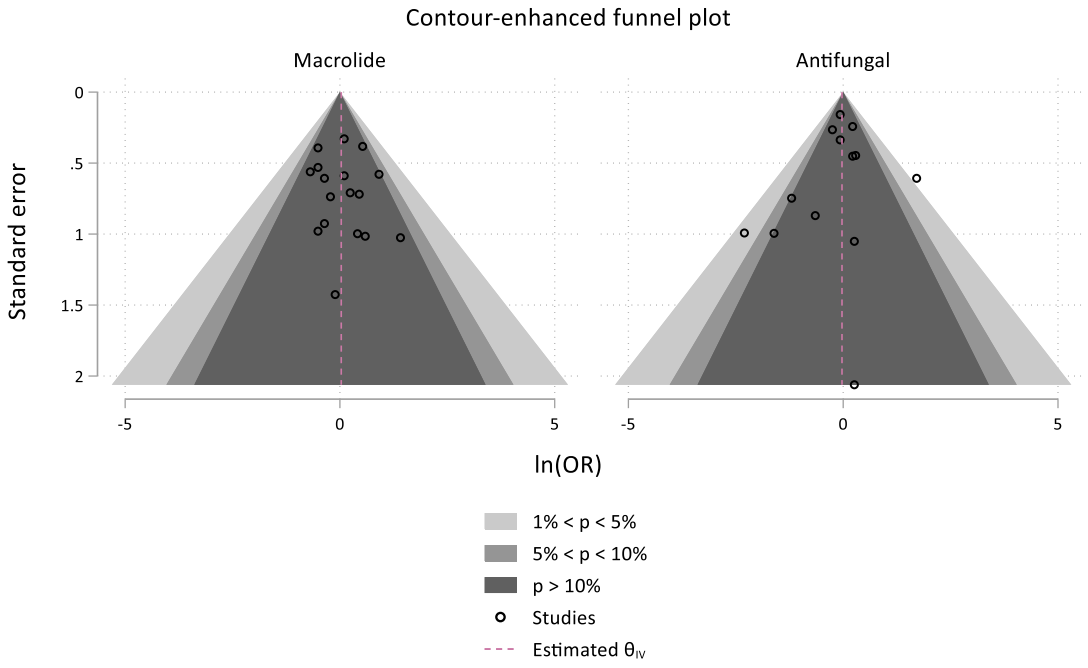

Figure 10 Funnel plots illustrating the publication bias of studies 1, 4, and 5 (macrolides) and studies 6, 7, 10, 11, and 13 (antifungals) in the meta-analysis.

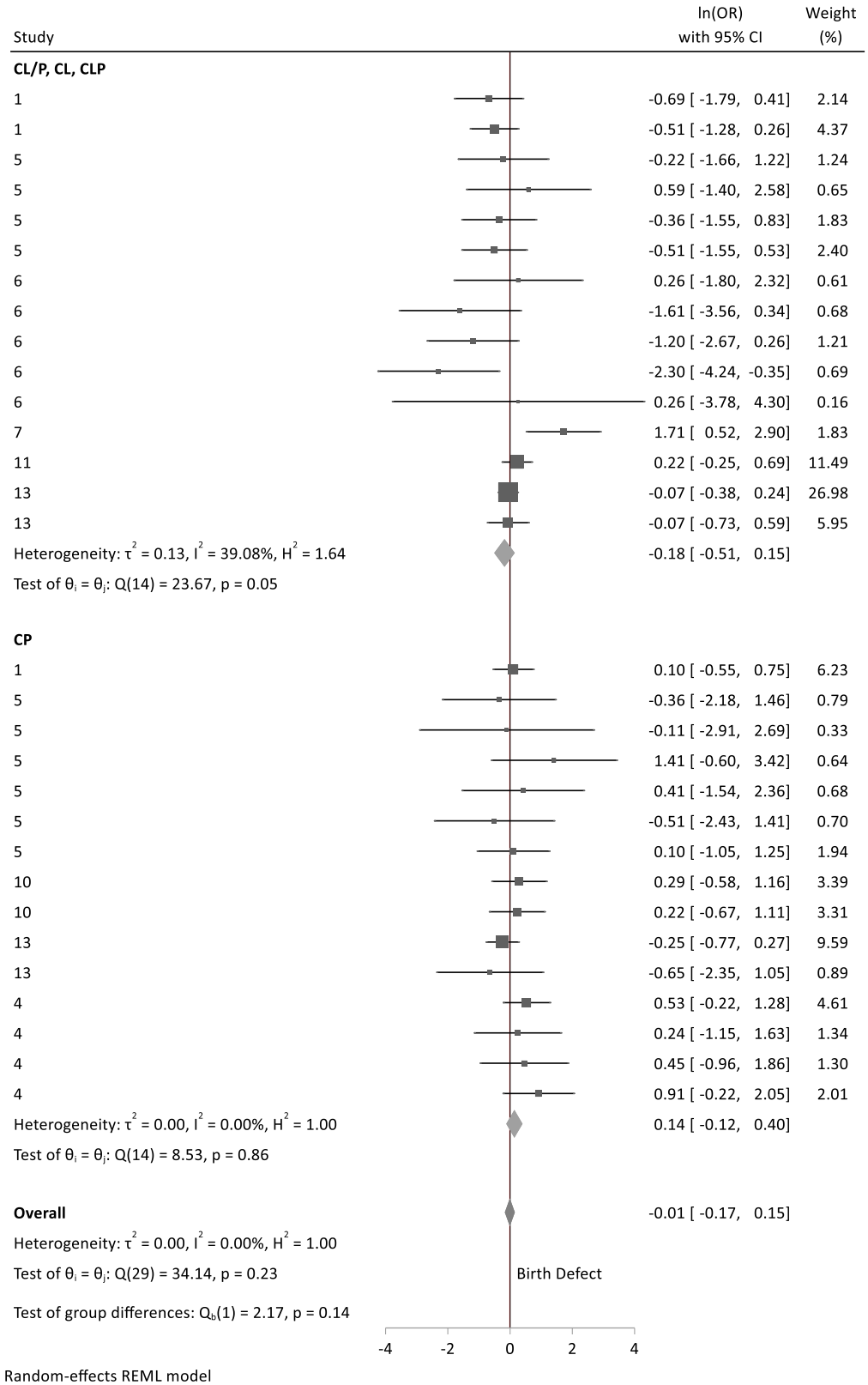

Figure 11 Forest plots of studies reporting the log (ln) of the OR and 95% CI of CL/P and CP (*i.e.* OR of 1 = chance of event or outcome = 0 on the Forest plot). The upper forest plot shows the OR of CL/P, CL, and CLP (studies 1, 5, 6, 7, 11, and 13), and the lower forest plot shows the OR of CP (studies 1, 4, 5, 10, and 13). In both cases the diamond crosses the line of no effect.

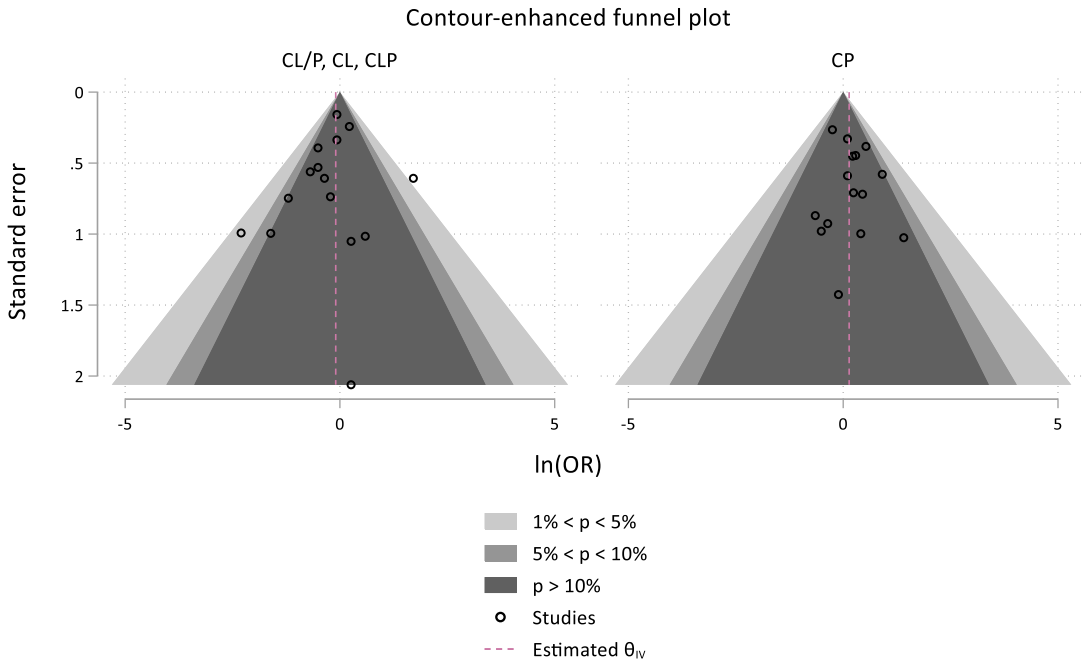

Figure 12 Funnel plots illustrating the publication bias of studies 1, 5, 6, 7, 11, and 13 (CL/P) and studies 1, 4, 5, 10, and 13 (CP) in the meta-analysis.

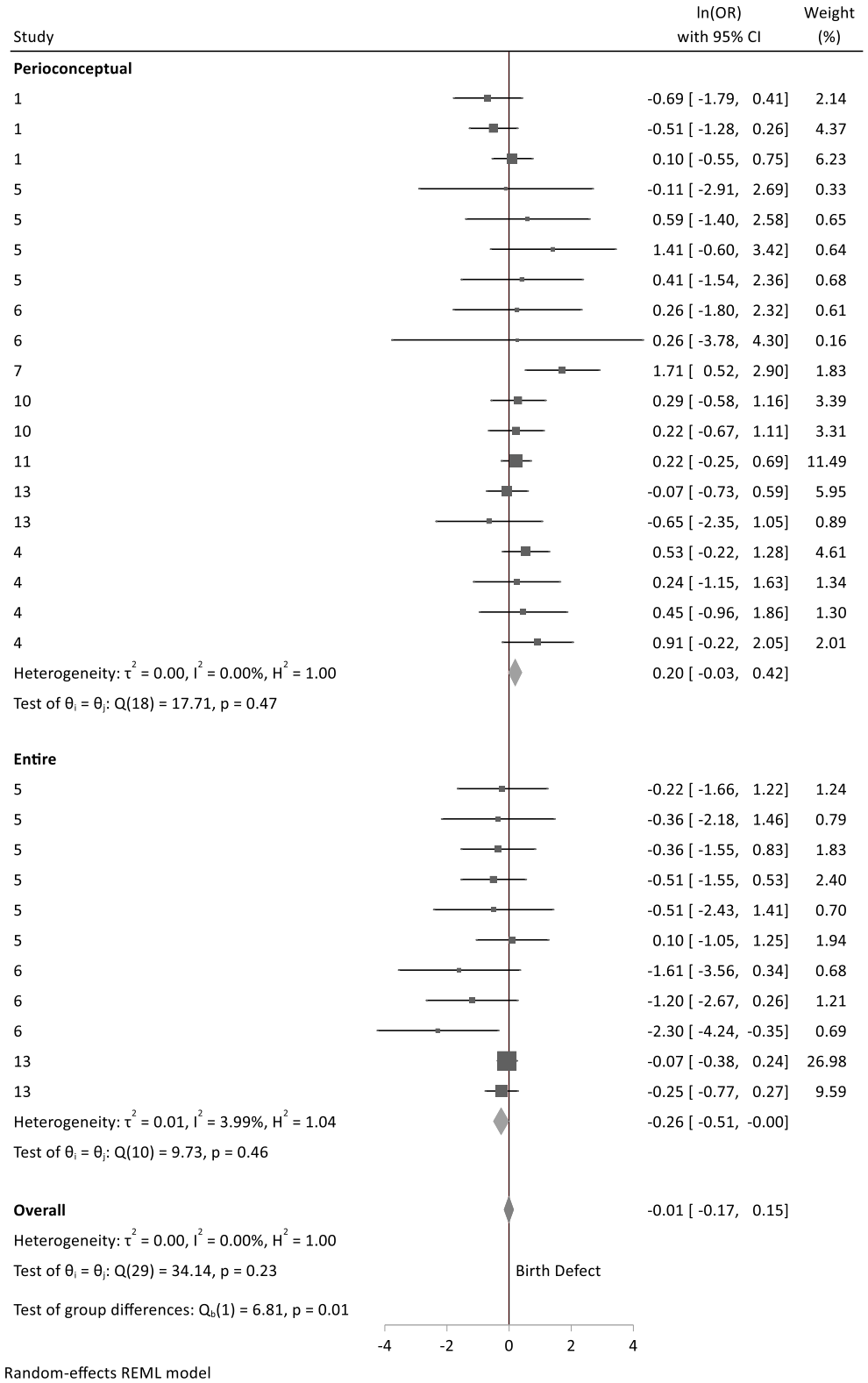

Figure 13 Forest plots reporting the log (ln) of the OR and 95% CI of cleft at different time exposures (*i.e.* OR of 1 = chance of event or outcome = 0 on the Forest plot). The upper forest plot shows the OR of cleft when exposed to macrolides/antifungals during the periconceptual period of pregnancy (studies 1, 4, 5, 6, 7, 10, 11, and 13), and the lower forest plot shows the OR of cleft when exposed to macrolides/antifungals during the entire pregnancy (studies 5, 6, and 13). In both, the diamond crosses the line of no effect.

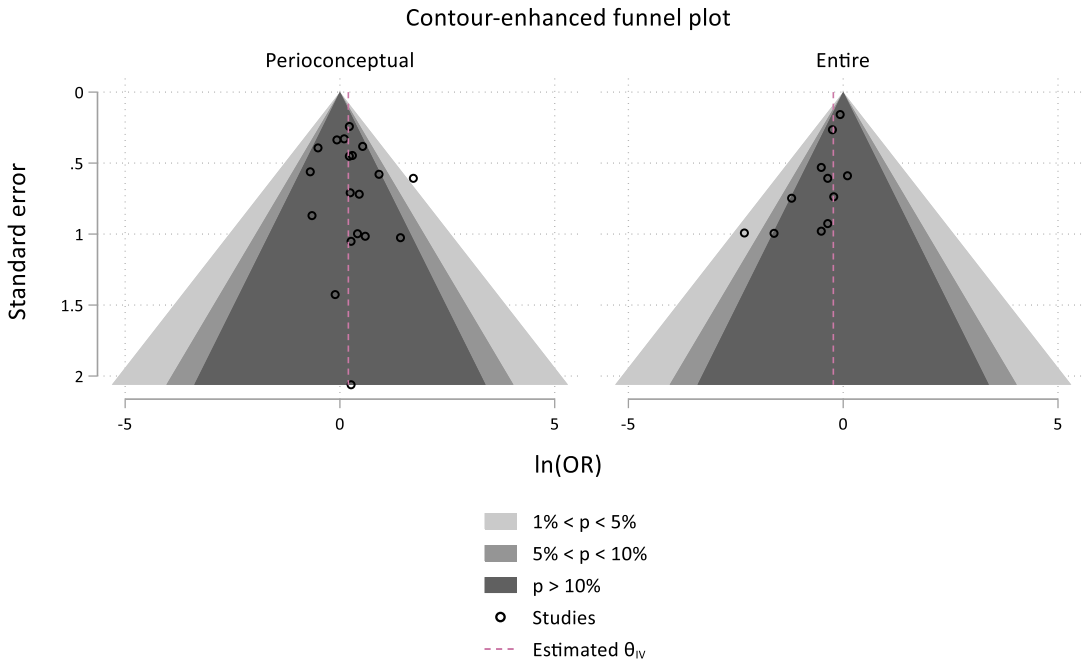

Figure 14 Funnel plots illustrating the publication bias of studies 1, 4, 5, 6, 7, 10, 11, and 13 (periconceptual) and studies 5, 6, and 13 (entire pregnancy) in the meta-analysis.

5.0 DISCUSSION

Reviews provide a summary of published literature on a specific topic. The two types of reviews in clinical research are systematic reviews and non-systematic or narrative reviews. Unlike systematic reviews, which follow a set of guidelines known as Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), narrative reviews do not (Liberati *et al*., 2009). The lack of methodologies makes narrative reviews more prone to limitations, some of which include (Mulrow, 1987):

- Subjectivity
- Methods not transparent
- Results not reproducible
- No quantitative summary
- Uncertainty

The principal differences between narrative and systematic reviews are shown in Table 10.

Conducting a systematic review is a time consuming and challenging task. Nevertheless, it is necessary in order to evaluate the efficiency of diagnostic and treatment interventions, as well as in this case, to help determine the outcome of therapeutic exposures (Collins and Fauser, 2005). As Sir Iain Chalmers, cofounder of the Cochrane Collaboration, states,

"……. the results of a particular research study cannot be interpreted with any confidence unless they have been synthesised, systematically, with the results of all other relevant studies.

… Science is meant to be cumulative, but researchers usually don't cumulate scientifically…" (Chalmers *et al.*, 2002)

	Narrative Reviews	Systematic Reviews
Main Features	• Describe and appraise published articles with no clear method for selection of articles.	• Well defined question. • Clearly defined criteria for selection of articles. • Explicit methodology for screening, extraction and synthesis of data. • Assess quality of studies.
Uses	• General debates, appraisal of previous studies and the current lack of knowledge. • Rationales for future research. • Speculate on new types of interventions available.	• Identify and analyse the literature on a specific question to identify the basis of that knowledge. • Rational, assumptions and methods are open to external examination due to explicit methodology and comprehensive analyses.
Limitations	• The assumptions and processes are often not known. • Selection and evaluation biases are not known. • Not reproducible.	• The scope is limited by the question, search terms, and the inclusion criteria. • The reader usually needs to come up with alternative questions that need to be answered.

Table 10 The principal differences between narrative and systematic reviews. (Source: Ferrari, 2015)

Bias is any process that interferes with producing results that deviate from the true values. Although systematic reviews aim at reducing bias and random error and employ an effective research methodology, systematic reviews still have several limitations, including the fact they are written retrospectively and so are prone to bias. This next section discusses the strengths and limitations of the current systematic review as a whole and of the individual included studies.

5.1 Strengths and Limitations of the Systematic Review and Meta-Analysis

The main objective of this systematic review and meta-analysis, as with any, was to formulate a well-defined question and provide qualitative and quantitative analyses based on the evidence available in the literature. The true teratogenic effects of antimicrobials in the development of cleft is still not well understood. Therefore, the aim of the review was clearly stated as "to investigate if there is any association between the occurrence of NSOFC and foetal exposure to macrolides, antifungals, and antivirals/ antiretrovirals *in utero* ". In addition to formulating a 'focused' question, the question also specifies the condition or diagnosis, the exposure, and the outcome, which are some of the criteria identified by Gregg *et al.* (2008) for assessing quality and strengths of a systematic review and meta-analysis.

This well-defined question set a foundation for the methodological process of the research. It had to be objective, reliable, and reproducible. The inclusion and exclusion criteria for the retrieval of studies were clearly listed, setting the boundaries for the review (vide infra 3.5.1 and 3.5.2). Three electronic databases (MEDLINE, EMBASE, and Cochrane CENTRAL) were searched and reference lists of retrieved articles were hand searched, as well. To minimise human error, the articles were selected in duplicates and disagreements were resolved by a third reviewer. This extensive process of identifying and selecting relevant studies implies that publication bias is reduced. However, it was not completely avoided as only published articles in the English language were included in the search. It may also be argued that a limitation of this systematic review is the limited number of databases included in the search. However, MEDLINE and EMBASE are the two key international biomedical databases available. Table 11 illustrates the details of both databases.

MEDLINE	EMBASE
U.S. National Library of Medicine	European, Elsevier
Biomedicine and health	Biomedicine, good for pharma research
Citations from > 5,600 journals 40 languages	Citations from > 8,500 journals 30 languages
> 23 million records updated daily	>30 million records updated daily (> 6 million records not on MEDLINE)
80% with English abstracts	80% with English abstracts
Searchable back to 1946	Searchable back to 1974

Table 11 Key sources: MEDLINE and EMBASE. (Source: Dawson, June 2018)

The PRISMA flow chart of the study selection was clearly presented, and all excluded studies were accounted for with the reason for exclusion (Moher *et al*., 1994) (Appendix D). A quality assessment checklist was obtained from the online CASP and was used to assess the quality of each included study (Tables 8 and 9). Risk of bias was also used to assess the level of bias in individual studies included in the review (Figures 5 and 6). Data was accumulated and analysed, and a meta-analysis was conducted. Due to heterogeneity across some of the articles, not all studies included in the review were included in the meta-analysis.

The protocol of this systematic review and meta-analysis was not registered in the International Prospective Register of Systematic Reviews (PROSPERO), an online database of systematic reviews currently being undertaken. It is recommended to register the review prior to commencing the search to avoid any duplications. It is also useful for recording progress of the review. By not registering this review onto PROSPERO, the risk of bias increases due to a potential lack of transparency in the review process.

Table 12 lists the strengths and limitations of this systematic review and meta-analysis.

Strengths	Limitations
• The aim was clearly stated. • The inclusion and exclusion criteria were stated. • Electronic databases (MEDLINE, EMBASE, and Cochrane CENTRAL) and reference lists of retrieved articles were searched. • Study selection and methodology was described. • Article selection was done in duplicates, and any disputes were resolved by a third reviewer. • List of included studies were provided. • Flow chart of study selection was provided. • Reason for exclusion of articles was given. • Quality assessment was done using CASP • Risk of bias was assessed (ROBINS-I). • Characteristics of individual studies were provided. • Meta-analysis was conducted. • Publication bias was assessed using Funnel Plots. • No conflicts of interest were reported.	• Protocol was not registered on the PROSPERO database. • Only published articles were included. • Foreign language articles were excluded. • Data extraction was not done in duplicates.

Table 12 Strengths and limitations of systematic review and meta-analysis.

5.2 Strengths and Limitations of the Included Studies

Critical appraisal is the process of judging the validity and quality of an article. Each study is critiqued according to the following (Derish and Annesley, 2011):

- Methodology used to test the hypothesis
- Limitations
- Validity and quality of results obtained
- Interpretation of the results

- Impact of the conclusion

For this systematic review, each article was critically appraised using the online CASP according to the type of study design implemented (Appendix C). The CASP quality assessment checklist addresses three questions:

1. Are the results of the study valid?
2. What are the results?
3. Will the results help locally?

The discussion of the strengths and limitations of the studies included in the review were addressed according to these three questions and are as follows:

5.2.1 Macrolides

Are the results of the study valid?

All five studies included in the review with macrolides as an exposure addressed a clearly focused issue, which included the population, exposure, and the outcome. The authors also used an appropriate study design to answer the question at hand.

Selection bias may compromise the validity of the findings, and therefore assessing whether the cases and controls were selected in an appropriate manner is crucial. All cases were recruited in an acceptable way from national birth registries, which included all pregnancies. Excluding eligible cases from the study increases the risk of selection bias. For example, some studies excluded still born and/or induced abortions (Källén *et al.*, 2005; Mølgaard-Nielsen and Hviid, 2011; Muanda *et al.*, 2017). This may have underestimated the true risk of cleft following maternal exposure to macrolides.

Two of the three case control studies included in this section of the review selected the controls randomly (Crider *et al.*, 2009) and matched them to the cases (Czeizel *et al.*, 1999), minimising selection bias. The controls were also representative of the population geographically, demographically, and temporally. The third case-control study selected the controls in a different manner. Källén *et al.* (2005) selected controls that were exposed to another type of antibiotic, penicillin V, which is a known non-teratogenic drug. Having cases and controls both exposed to antibiotics reduced the risk of confounding bias, as all participants had an underling infection that needed antibiotic treatment.

In terms of confounding, all included studies in the review considered the potential confounding factors in the analysis and were clearly listed. Some of which included maternal age, ethnicity, parity, education, BMI, smoking, maternal disorders, periconceptional smoking and alcohol use, the consumption of folic acid, multivitamins, and other drugs during pregnancy. Although potential confounders have been adjusted for, residual or unmeasured confounding cannot be completely ruled out. For example, Muanda *et al.* (2017) did not account for maternal smoking, alcohol consumption, and folic acid intake, which have been shown to increase the risk of CLP.

Recall bias is another factor to consider when critically appraising an article as it too may affect the findings. Mølgaard-Nielsen and Hviid (2011) and Muanda *et al.* (2017) measured the exposure objectively. Exposure data was taken from a public prescription drug insurance registry and from prescriptions and physician records, respectively. This minimised the risk of recall bias as well as detection bias. However, it may have increased the risk for information bias as not all mothers may have been compliant with taking the prescribed medication. The remaining studies obtained their exposure data subjectively by interviewing

the mothers either on the phone or by filling out a questionnaire. The time at which exposure data was collected varied from as early as 10-12 weeks of gestation, to as late as 24 months postpartum. This may have increased the risk of recall and information bias as they may not fully or accurately recall the exact drug name and at what time during pregnancy it was taken.

What are the results?

Attempting to understand whether the results are believable relies on the level of bias. In observational studies, residual or unmeasured confounding factors are a key issue. Elevated OR may have occurred due to underlying maternal conditions and unmeasured confounding factors. This may result in overestimation of the outcome and a false association between the exposure and outcome. For example, maternal smoking increases the risk of respiratory infections leading to high intake of antibiotics. Some of the analyses investigating the association between macrolides and clefts were underpowered because of the small number of exposed cases (Crider *et al.*, 2009; Czeizel *et al.*, 1999).

Will the results help locally?

The results of the included studies in this review agree with the results of previously published studies in that there is no increased risk of cleft development when exposed to macrolides *in utero*. To be critical, the results should only be applied with confidence to mothers of the same geographical region the study was conducted in, as geography may play a major role in the prevalence of CLP (Mossey *et al*., 2011).

5.2.2 Antifungals

Are the results of the study valid?

The eight studies included in this section of the review all addressed a well-defined question. Cases were recruited in an acceptable way either from national birth registries or congenital malformation registries. One limitation of most of the studies is that they were only based on live births. By excluding terminated pregnancies, bias increases towards the null hypothesis because of the possibility of excluding pregnancies that may have been terminated due to the presence of a congenital malformation. Of the eight studies, two studies included stillbirths and/or elective terminations (Carter *et al.*, 2008; Nørgaard *et al.*, 2008). Controls were also recruited with minimum bias and were representative of the defined population. Controls were either selected randomly from the same geographic area as the cases and around the same time, or they were matched according to gender, birth week, and geographic region.

Overall, exposures were clearly defined and accurately measured with minimum bias. Studies included in this section of the review that measured the exposure objectively strengthened the study, and those that were measured subjectively weakened the study. Exposure data was extracted either from medical records, questionnaires and interviews, or a combination of both. By using questionnaires and interviewing mothers, the risk of recall and information bias increases (Howley *et al.*, 2016; Carter *et al.*, 2008; Czeizel *et al.*, 1999). Interviewing mothers within 24 months of delivery increases the risks of mothers failing to recall the medication used, the dose, route of administration and duration of use. Antifungals are usually used for a short period of time of around seven to ten days and therefore, these types of details are easily forgotten. This potential under reporting of exposure may have contributed to the small number of exposed cases in these studies and therefore small OR. Reporting exposure objectively from medical records and prescription databases also comes with limitations. Firstly, we do not know whether these mothers took

the medication or not, and secondly, there is no data on 'over the counter' medication or medications prescribed by other practitioners (Hill *et al.*, 1988; Jick, 1999; Mølgaard-Nielsen *et al.*, 2013; Nørgaard *et al.*, 2008). Czeizel *et al.* (2003) avoided these limitations by measuring exposure from medical records, the antenatal care logbook registry which records all prescribed drugs, and from questionnaires to the mothers. This allows for good triangulation of the data.

Potential confounding factors were accounted for in the design and analysis of most of the studies in this section. However, unmeasured confounding is always a risk for false association between exposure and outcome. Was the development of a cleft due to the fungal infection itself, or was it due to the antifungal medication used to treat the infection that was the cause? This is referred to as indication bias. Maternal illness, a potential confounding factor, was not accounted for in the study by Mølgaard-Nielsen *et al.* (2013) as the registry they recruited their cohort from did not include this important information. Two of the eight studies did not state whether they had accounted for confounding factors (Hill *et al.*, 1988; Jick, 1999). They may have not accounted for smoking, which is known to be associated with cleft development, or they may have not taken older maternal age into consideration, which is also linked with an increased risk of cleft.

What are the results?

Across the studies included in this review, the authors found no significantly increased risk of CLP when exposed to fluconazole *in utero*. These results were consistent with results of previous studies. However, the small number of exposed infants in the birth defect groups, including CLP, made it challenging to calculate a risk estimate. It may also be worthwhile to state that measuring exposure a month before pregnancy may have biased the estimates

towards the null hypothesis, as the embryo may not have developed during that time. The study by Howley *et al.* (2016) was the only study that found an increased risk of CLP with infants exposed to fluconazole periconceptionally. This association has not been reported in previous observational studies. Since there was no data on dose, the results may have been impacted by including high dose users into the cohort. As with the remaining studies, which found no evident association with CLP and topical econazole (Czeizel *et al.*, 2003) or clotrimazole (Czeizel *et al.*, 1999), no definite conclusion can be made as they were single case control studies assessing different antifungal medications.

Will the results help locally?

The results are reassuring. However, although the current studies show no increased risk of congenital malformations when exposed to antifungal medication during the critical period of pregnancy, the authors suggested larger cohorts of exposed pregnant women are needed to rule out the true association between fluconazole during early pregnancy and specific birth defects, such as CLP. A recent publication from the United States of America examined nearly two million pregnancies of which 38,000 were exposed to oral fluconazole in the first trimester (Zhu *et al*., 2020). This was not associated with OC but there was an association with musculoskeletal malformations which corresponded to a small adjusted risk difference of about 12 incidents per 10,000 exposed pregnancies overall.

5.2.3 Antivirals/ Antiretrovirals

Are the results of the study valid?

This section of the review included four studies, all of which had clearly stated aims and objectives with the appropriate study design to tackle the question posed. However, because they were retrospectives studies, they are most likely at risk of bias. The cohorts

were recruited in an acceptable way and were representative of the targeted population. They were recruited from the FDA's Adverse Events Reporting System database (Cartsos *et al.*, 2012), the National Study of HIV in Pregnancy and Childhood (Townsend *et al.*, 2009), and from outpatient clinics of the Beijing Ditan Hospital (Liu *et al.*, 2013). However, the study conducted by Minakami *et al.* (2014) was prone to recall and selection bias. Physicians were asked to provide information on all women who they prescribed laninamivir to during pregnancy. Only half of the physicians participated in the study. This selective participation may have been affected by either a positive or adverse experience with the drug in question. Having not included any controls may be a limitation of these four observational studies, as well. However, risks were compared to that of the general population, and it was thought to be adequate for the analysis.

The aetiology of CLP is multifactorial, *i.e*. both genetic and environmental factors play a role. Therefore, unaccounted confounding factors may overestimate the risks and result in false associations between the exposure and the outcome. Mothers with HIV have characteristic lifestyles that make them more prone to delivering infants with congenital malformations, and therefore the results and subsequent conclusions made by the authors must be interpreted with caution (Cartsos *et al.*, 2012; Townsend *et al.*, 2009).

What are the results?

Sample size has a significant impact on the interpretation of results. Having a small cohort to study may underestimate the association between exposure and outcome. For example, Minakami *et al.* (2014) recruited only 112 pregnant women of whom three gave birth to infants with congenital malformations, only one of which was CL. Although the results were

inconclusive, they do provide some level of reassurance for mothers being treated with laninamivir during pregnancy.

Confidence intervals are another factor to assess when interpreting results of studies. Having a wide CI is one limitation of a study and is usually associated with a small sample size. This was the case with the study by Cartsos *et al.* (2012), which had the widest CI ranging from 85.89 to 447.32 for efavirenz as the exposure.

Will the results help locally?

One author concluded that there was a potential association between *in utero* antiretroviral use and cleft (Cartsos *et al.* 2012), whilst the other concluded there was no such association (Townsend *et al.*, 2009). However, with the limited evidence available, the vast heterogeneity that exists between studies, and the limitations presented above, further research is required to reach a valid conclusion on the safety of antiretroviral drugs taken periconceptually. With respect to antivirals, the authors concluded that there was no increased risk of cleft development with their use during pregnancy. However, due to the small sample size and wide CIs, the results should be interpreted with caution (Cartsos *et al.* 2012).

In summary, the strengths of the included studies were as follows:

- Addressed a well-defined question
- Cases and controls recruited in an acceptable way
- Exposure measured to minimise bias
- Potential confounders accounted for
- Believable and applicable results

The limitations of the included studies were:

- Biases (*e.g.* selection, recall, information, confounding and publication bias)
- Lack of assessment of these biases
- Lack of causality
- Heterogeneity

5.3 Recently Published Systematic Reviews and Meta-Analyses

To round off this review, the electronic databases (MEDLINE, EMBASE, and Cochrane library) were searched for the latest published systematic reviews and meta-analyses on the adverse effects of macrolides, antifungals, and antivirals/ antiretrovirals when taken during pregnancy. Four relevant papers were retrieved. They were published in the year 2019 and 2020.

1. Wu *et al.*, 2020. Efficacy and safety of antiviral therapy for HBV in different trimesters of pregnancy: Systematic review and meta-analysis.
2. Liu *et al.*, 2019. Foetal outcome after maternal exposure to oral antifungal agents during pregnancy: A systematic review and meta-analysis.
3. Zhang *et al.*, 2019. The safety of oral fluconazole during the first trimester of pregnancy: A systematic review and meta-analysis.
4. Fan *et al.*, 2019. Associations between use of macrolide antibiotics during pregnancy and adverse child outcomes: A systematic review and meta-analysis.

5.3.1 Wu *et al.*, 2020

Several antiviral medications, such as lamivudine, telbivudine, and tenofovir have been approved by the FDA and have been shown to be effective and safe in preventing mother to

child transmission of HBV. However, there is no current evidence on the most effective and safe time of administering the antiviral therapy during pregnancy. Therefore, the aims and objectives of this systematic review were to identify the most effective time to start antiviral therapy (early-middle pregnancy or late pregnancy) and to assess the foetal outcomes at different gestational intervals. PubMed, EMBASE, Web of Science, and Cochrane databases were searched for studies that assess the efficacy and safety of antiviral therapy during pregnancy up to July 1, 2019. Three randomised and 32 nonrandomised controlled trials were included in the review. The meta-analysis confirmed that administration of antiviral therapy prior to week 28 of gestation was associated with a lower risk for mother to child transmission of HBV than when administered later in pregnancy. There were no significant differences in the adverse effects of mothers and infants according to the time of administration of the antiviral. Unfortunately, no data was available on whether the type and time of administration of the antiviral influenced the development of CLP.

5.3.2 Liu *et al.*, 2019

Vulvovaginal candidiasis occurs commonly during pregnancy and topical antifungals are usually prescribed. However, when topical antifungals fail, oral antifungals are prescribed. Since the foetal safety of oral antifungals remains debatable, this systematic review and meta-analysis aimed to investigate this dilemma. PubMed, EMBASE, and Cochrane Central Register of Controlled Trials (CENTRAL) were searched up to October 31, 2018. Nine studies matched the inclusion and exclusion criteria. Eight were cohort studies and the other was a case control study. The authors concluded that *in utero* exposure to fluconazole or itraconazole is not associated with an increased risk of birth defects in general. However, it is associated with an increased risk of specific birth defects. For example, oral fluconazole

increases the risk of limb and congenital heart defects, and oral itraconazole increases the risk of eye defects. Regarding CLP, only one case control study by Howley *et al.* (2016) found a significant association with fluconazole exposure during the first trimester as described in the current review.

5.3.3 Zhang *et al.*, 2019

This was another systematic review and meta-analysis investigating the safety of oral fluconazole during the first trimester of pregnancy and published less than two months before the systematic review by Liu *et al.* (2019). PubMed, MEDLINE, EMBASE, Cochrane Library, ClinicalTrials.gov, and the metaRegister of Controlled Trials were searched up to April 2019. Six cohort studies and one case control study fitted the inclusion and exclusion criteria. The results suggested that oral fluconazole use during the first trimester is associated with spontaneous abortions and congenital heart defects. There was no mention of cleft as an adverse foetal outcome in this review.

5.3.4 Fan *et al.*, 2019

Being the most used group of antibiotics worldwide, it is crucial to understand the potential adverse effects of macrolides when taken during pregnancy. As the current evidence is inconsistent, this systematic review and meta-analysis was conducted to investigate the association between *in utero* macrolide exposure and the adverse effects on both the foetus and the infant. Electronic databases were searched up to February 15, 2018. These included PubMed, EMBASE, Cochrane Library, Conference Proceeding Citation Index-Science, and ClinicalTrials.gov. Nineteen studies were included in the review: ten observational studies and nine randomised controlled trials. The study showed an increased risk of miscarriage when pregnant mothers were exposed to macrolides. However, its association with cerebral

palsy, epilepsy, and gastrointestinal malformations was inconsistent. Moreover, there was insufficient evidence regarding other congenital malformations, including CLP, to conclude any definite association.

6.0 CONCLUSION

Interpreting, analysing, and critically appraising the current research was a challenging task. Studies investigating the efficacy and safety of drugs are usually associated with ethical precautions. Hence, all the studies that were included in this systematic review and meta-analysis were observational studies rather than randomised controlled trials and comprised both cohort and case control studies. Since most of the studies were retrospective in design, causal relationships are impossible to determine and only associations between exposure and outcome can be deduced. Therefore, the intention of this research was to systematically review all relevant published studies up to April 30, 2019, and to investigate any association between maternal exposure to antimicrobials (macrolides, antifungals, and antivirals/ antiretrovirals) and the development of NSOFC.

The review and its pooled analysis showed no overall increased risk of NSOFC associated with *in utero* exposure of macrolides, antifungals, and antivirals/ antiretrovirals. On this basis, the null hypothesis can be accepted. There was also very little effect of time of exposure on the risk of an adverse outcome. Reassuringly, our review supports the published literature on the safety of erythromycin and fluconazole in low therapeutic doses, the most widely used macrolide and antifungal respectively, among pregnant women. With the antivirals and antiretrovirals, no overall conclusion can be reached as the data was limited and heterogenous, and so could not be included in the meta-analysis.

However, it can be argued that the overall results provide a degree of reassurance, in that there were very few increases in the OR associated with mothers who were exposed to the antimicrobials in the included studies. This may have been due to limiting factors, such as indication for use or simply by chance. Although the risk may have been small, physicians

should consider prescribing safer alternative medications to treat the maternal infection wherever possible *e.g*. penicillin V rather than a macrolide. If not, it is imperative that pregnant mothers are aware of the potential adverse risks associated with using these antimicrobials during their pregnancy. Mothers exposed to macrolides, antifungals, and antivirals/ antiretrovirals should also be educated about undergoing a routine anomaly scan for early detection of CLP. Moreover, healthcare workers, including practitioners and nurses, should be trained to examine babies for the presence of OFC at the time of delivery.

7.0 FUTURE WORK

This systematic review and meta-analysis has hopefully set a foundation for future studies to clarify the true association between maternal intake of macrolides, antifungals, and antivirals/ antiretrovirals and the development of NSOFC in children. Given the multifactorial aetiology of CLP, further investigations are recommended with larger numbers of mothers recruited and with a greater emphasis on CLP as the main adverse foetal outcome in the study. This knowledge will increase awareness of the potential risks of antimicrobials among pregnant women and women of child-bearing age worldwide.

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APPENDICES

Appendix A – Electronic Search Strategy

Macrolides

Search	MEDLINE	Embase
1. ("cleft lip" or "cleft lips" or "cleft palate" or "cleft palates" or "orofacial cleft" or "orofacial clefts").mp.	28258	28519
2. pregnan*.mp.	966816	833820
3. 1 AND 2	3534	3895
4. (antibiotic* or macrolide* or azithromycin* or clarithromycin* or erythromycin* or fidaxomicin* or telithromycin*).mp.	391031	757387
5. 3 AND 4	13	104

Antifungals

Search	MEDLINE	Embase
1. ("cleft lip" or "cleft lips" or "cleft palate" or "cleft palates" or "orofacial cleft" or "orofacial clefts").mp.	28258	28519
2. pregnan*.mp.	966816	833820
3. 1 AND 2	3534	3895
4. (antigungal* or fluconazole* or diflucan* or griseofulvin* or gris peg* or fulvicin pg* grifulvin v* or itraconazole* or sporanox* or ketoconazole* or nizarol* or terbinafine* or lamisil*).mp.	31131	158453
5. 3 AND 4	10	35

Antivirals/ Antiretrovirals

Search	MEDLINE	Embase
1. ("cleft lip" or "cleft lips" or "cleft palate" or "cleft palates" or "orofacial cleft" or "orofacial clefts").mp.	28258	28519
2. pregnan*.mp.	966816	833820
3. 1 AND 2	3534	3895
4. (antiviral* or amantidine* or symmetrel* or rimantadine* or flumadine* or oseltamivir* or tamiflu* or zanamivir* or relenza*).mp.	127950	168787
5. 3 AND 4	2	7

Appendix B – Data Extraction Form

1. GENERAL INFORMATION

Name/ID of data extractor	
Date data extraction completed *(dd/mm/yyyy)*	
Title	
First author	
Year of publication	
Journal published in	
Publication type *(e.g. journal article, abstract, book chapter, etc.)*	
Country of study	
Study funding source *(including role of funders)*	

2. STUDY ELIGIBILITY

		Page
Type of study *(e.g. case control, cohort, etc.)*		
Setting		
Participants		
Include/Exclude		

3. STUDY DETAILS

		Page
Enrolment and follow-up period *(start and end date of study)*		
Length of follow-up		
Aim of study		

4. METHODS

		Page
Method/s of recruitment of participants		
Inclusion criteria		
Exclusion criteria		
Exposure		
Representativeness *(is the sample representative of the target population)*		
Sample size calculation *(what assumptions were made?)*		
Randomisation		
Unit of analysis *(e.g. by individuals, health professional, practice, hospital, community)*		
Statistical methods used *(e.g. descriptive, inferential, etc.)*		

5. RESULTS

		Page
Number of participants		
Number allocated to each intervention group		
Were participants who entered the study adequately accounted for?		
Percentage of participants who completed the study		
Percentage of participants who received the intervention/exposure		
Number of participants with missing information		
Age *(median, mean, range)*		
Sex		
Race/Ethnicity		
Other sociodemographic factors *(e.g. educational level, literacy level, socio-economic status, etc.)*		
Co-morbidities		

6. OUTCOMES

Page

Comparison		
Outcome		
Time points measured *(specify whether from start or end of intervention)*		
Person measuring/ reporting		
Blinding		
Unit of measurement *(if relevant)*		
Scales: upper and lower limits *(indicate whether high or low score is good)*		
Imputation of missing data *(e.g. assumptions made for ITT analysis)*		
Assumed risk estimate *(e.g. baseline or population risk noted in Background)*		
Outcomes specific to CLP		

7. APPLICABILITY

Have important populations been excluded from the study? *(consider disadvantaged populations, and possible differences in the intervention effect) Yes/No/Unclear*	
Is the intervention likely to be aimed at disadvantaged groups? *(e.g. lower socioeconomic groups) Yes/No/Unclear*	
Does the study directly address the review question? *(any issues of partial or indirect applicability) Yes/No/Unclear*	
Key conclusions of study authors	
References to other relevant studies	

Appendix C – CASP Quality Assessment Checklist

Cohort

Section A: Are the results of the study valid?

1. Did the study address a clearly focused issue?

☐ Yes

☐ Can't Tell

☐ No

HINT: A question can be 'focused' in terms of

- The population studied
- The risk factors studied
- Is it clear whether the study tried to detect a beneficial or harmful effect?
- The outcomes considered

2. Was the cohort recruited in an acceptable way?

☐ Yes

☐ Can't Tell

☐ No

HINT: Look for selection bias which might compromise the generalisability of the findings:

- Was the cohort representative of a defined population?
- Was there something special about the cohort?
- Was everybody included who should have been?

Is it worth continuing?

3. Was the exposure accurately measured to minimise bias?

☐ Yes

☐ Can't Tell

☐ No

HINT: Look for measurement or classification bias:

- Did they use subjective or objective measurements?
- Do the measurements truly reflect what you want them to (have they been validated)?

- Were all the subjects classified into exposure groups using the same procedure?

4. Was the outcome accurately measured to minimise bias?

☐ Yes

☐ Can't Tell

☐ No

HINT: Look for measurement or classification bias:

- Did they use subjective or objective measurements?
- Do the measurements truly reflect what you want them to (have they been validated)?
- Has a reliable system been established for detecting all the cases (for measuring disease occurrence)?
- Were the measurement methods similar in the different groups?
- Were the subjects and/or the outcome assessor blinded to exposure (does this matter)?

5. (a) Have the authors identified all important confounding factors?

☐ Yes

☐ Can't Tell

☐ No

HINT:

- List the ones you think might be important, and ones the author missed

5. (b) Have they taken account of the confounding factors in the design and/or analysis?

☐ Yes

☐ Can't Tell

☐ No

HINT:

- Look for restriction in design, and techniques *e.g.* modelling, stratified-, regression-, or sensitivity analysis to correct, control or adjust for confounding factors

6. (a) Was the follow up of subjects complete enough?

☐ Yes

☐ Can't Tell

☐ No

HINT: Consider

- The good or bad effects should have had long enough to reveal themselves
- The persons that are lost to follow-up may have different outcomes than those available for assessment
- In an open or dynamic cohort, was there anything special about the outcome of the people leaving, or the exposure of the people entering the cohort?

6. (b) Was the follow up of subjects long enough?

☐ Yes

☐ Can't Tell

☐ No

Section B: What are the results?

7. What are the results of this study?

HINT: Consider

- What are the bottom line results?
- Have they reported the rate or the proportion between the exposed/unexposed, the ratio/rate difference?
- How strong is the association between exposure and outcome (RR)?
- What is the absolute risk reduction (ARR)?

8. How precise are the results?

HINT:

- Look for the range of the confidence intervals, if given

9. Do you believe the results?

☐ Yes

☐ Can't Tell

☐ No

HINT: Consider

- Big effect is hard to ignore
- Can it be due to bias, chance or confounding?
- Are the design and methods of this study sufficiently flawed to make the results unreliable?
- Bradford Hills criteria (*e.g.* time sequence, dose-response gradient, biological plausibility, consistency)

Section C: Will the results help locally?

10. Can the results be applied to the local population?

☐ Yes

☐ Can't Tell

☐ No

HINT: Consider whether

- A cohort study was the appropriate method to answer this question
- The subjects covered in this study could be sufficiently different from your population to cause concern
- Your local setting is likely to differ much from that of the study
- You can quantify the local benefits and harms

11. Do the results of this study fit with other available evidence?

☐ Yes

☐ Can't Tell

☐ No

12. What are the implications of this study for practice?

☐ Yes

☐ Can't Tell

☐ No

HINT: Consider

- One observational study rarely provides sufficiently robust evidence to recommend changes
- To clinical practice or within health policy decision making
- For certain questions, observational studies provide the only evidence
- Recommendations from observational studies are always stronger when supported by other evidence

Case Control

Section A: Are the results of the study valid?

1. Did the study address a clearly focused issue?

☐ Yes

☐ Can't Tell

☐ No

HINT: An issue can be 'focused' In terms of

- The population studied
- Whether the study tried to detect a beneficial or harmful effect the risk factors studied

2. Did the authors use an appropriate method to answer their question?

☐ Yes

☐ Can't Tell

☐ No

HINT: Consider

- Is a case control study an appropriate way of answering the question under the circumstances?
- Did it address the study question?

Is it worth continuing?

3. Were the cases recruited in an acceptable way?

☐ Yes

☐ Can't Tell

☐ No

HINT: We are looking for selection bias which might compromise validity of the findings

- Are the cases defined precisely?
- Were the cases representative of the defined population (geographically and/or temporally)?
- Was there an established reliable system for selecting all the cases?
- Are they incident or prevalent?
- Is there something special about the cases?
- Is the time frame of the study relevant to disease/exposure?

- Was there a sufficient number of cases selected?
- Was there a power calculation?

4. Were the controls selected in an acceptable way?

☐ Yes

☐ Can't Tell

☐ No

HINT: We are looking for selection bias which might compromise the generalisability of the findings

- Were the controls representative of the defined population (geographically and/or temporally)?
- Was there something special about the controls?
- Was the non-response high, could non-respondents be different in any way?
- Are they matched, population based or randomly selected?
- Was there a sufficient number of controls selected?

5. Was the exposure measured to minimise bias?

☐ Yes

☐ Can't Tell

☐ No

HINT: We are looking for measurement, recall or classification bias

- Was the exposure clearly defined and accurately measured?
- Did the authors use subjective or objective measurements?
- Do the measures truly reflect what they are supposed to measure (have they been validated)?
- Were the measurement methods similar in the cases and controls?
- Did the study incorporate blinding where feasible?
- Is the temporal relation correct (does the exposure of interest precede the outcome)?

6. (a) Aside from the experimental intervention, were the groups treated equally?

☐ Yes

☐ Can't Tell

☐ No

HINT: List the ones you think might be important, that the author may have missed

- Genetic
- Environmental
- Socio-economic

6. (b) Have the authors taken account of the potential confounding factors in the design and/or in their analysis?

☐ Yes

☐ Can't Tell

☐ No

HINT: Look for

- Restriction in design, and techniques (*e.g.* modelling, stratified-, regression-, or sensitivity analysis to correct, control or adjust for confounding factors)

Section B: What are the results?

7. How large was the treatment effect?

HINT: Consider

- What are the bottom line results?
- Is the analysis appropriate to the design?
- How strong is the association between exposure and outcome (look at the odds ratio)?
- Are the results adjusted for confounding, and might confounding still explain the association?
- Has adjustment made a big difference to the OR?

8. How precise was the estimate of the treatment effect?

HINT: Consider

- Size of the p-value
- Size of the confidence intervals
- Have the authors considered all the important variables?
- How was the effect of subjects refusing to participate evaluated?

9. Do you believe the results?

☐ Yes

☐ Can't Tell

☐ No

HINT: Consider

- Big effect is hard to ignore!
- Can it be due to chance, bias, or confounding?
- Are the design and methods of this study sufficiently flawed to make the results unreliable?

- Consider Bradford Hills criteria (*e.g.* time sequence, does-response gradient, strength, biological plausibility)

Section C: Will the results help locally?

10. Can the results be applied to the local population?

☐ Yes

☐ Can't Tell

☐ No

HINT: Consider whether

- The subjects covered in the study could be sufficiently different from your population to cause concern
- Your local setting is likely to differ much from that of the study
- Can you quantify the local benefits and harms?

11. Do the results of this study fit with other available evidence?

☐ Yes

☐ Can't Tell

☐ No

HINT: Consider

- All the available evidence from RCT's Systematic Reviews, Cohort Studies, and Case Control Studies as well, for consistency

Appendix D – Flow diagram showing study selection