**Table 1.** Signals for Macrolides identified in the literature up to 2016

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Macrolide studied** | **Signals Identified** | **Author/year** | **Study type** | **Exposed events** | **AOR/RR (CI)** |
| Macrolides | Congenital heart defect | Källén et al. 200310 | Case-control | 29 | **1.79 (1.3 - 2.8)** |
| Congenital anomalies of the genital organs | Lin et al. 2013.21 | Case-control | 6 | **2.8 (1.0 - 7.7)** |
| Erythromycin | Any malformation | Källén et al. 200511 | Cohort | 103 | **1.24 (1.0 - 1.5)** |
| Congenital heart defect | Källén et al. 201312 | Cohort | 43 | **1.70 (1.3 - 2.3)** |
| Anencephaly | Crider et al. 200917 | Case-control | 7 | **2.4 (1.1 - 5.3)** |
| Limb deficiency | Crider et al. 200917 | Case-control | 14 | **1.8 (1.0 - 3.3)** |
| Azithromycin | Congenital heart defect | Bar-Oz et al. 201220 | Cohort | 234 | 3.59 (0.99 – 12.9) |
|  | Orofacial clefts | Cooper et al. 200816 | Cohort | 2 | 4.9 (0.90 - 26.6) |

AOR: adjusted odds ratio; RR: relative risk; CI: confidence interval; Madeline was searched using the following MeSH and text terms combination: (‘Pregnancy’ OR all ‘text terms’) AND (‘Anti-Bacterial Agents’ OR all ‘antibiotic class/type’) AND (‘Congenital abnormality’ OR all ‘text terms’).

Table 2. Comparison of selected maternal characteristics between cases and control groups

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Maternal/fetal characteristics | Cases  n (%) | Non-genetic controls  n (%) | Chi-Squared  P-value | Genetic controls  n (%) | Chi-Squared  P-value |
| Total | **100702 (70.31)** | **23467 (16.38)** |  | **19060 (13.31)** |  |
| Maternal age group (years) |  |  | **≤0.001** |  | **≤0.001** |
| >20 | 4813 (4.78) | 941 (4.01) |  | 474 (2.49) |  |
| 20-24 | 19214 (19.08) | 3,844 (16.38) |  | 1846 (9.69) |  |
| 25-29 | 29947 (29.74) | 7,073 (30.14) |  | 3651 (19.16) |  |
| 30-34 | 26830 (26.64) | 6,822 (29.07) |  | 4562 (23.93) |  |
| 35-39 | 13049 (12.96) | 3,312 (14.11) |  | 4990 (26.18) |  |
| 40+ | 3059 (3.04) | 763 (3.25) |  | 3011 (15.80) |  |
| Unknown | 3790 (3.76) | 712 (3.03) |  | 526 (2.76) |  |
| Type of birth |  |  | **≤0.001** |  | **≤0.001** |
| Live birth | 95595 (94.93) | 22014 (93.81) |  | 12,992 (68.16) |  |
| Stillbirth/fetal deaths | 1072 (1.06) | 306 (1.30) |  | 466 (2.44) |  |
| TOPFA | 4033 (4.00) | 1147 (4.89) |  | 5,599 (29.38) |  |
| Not known | 2 (0.00) | 0 (0.00) |  | 3 (0.02) |  |
| Registry |  |  | **≤0.001** |  | **≤0.001** |
| Odense\_Denmark | 1,643 (1.63) | 338 (1.44) |  | 494 (2.59) |  |
| Tuscany\_Italy | 7,069 (7.02) | 1,843 (7.85) |  | 1,679 (8.81) |  |
| N Netherlands\_Netherlands | 4,719 (4.69) | 1,267 (5.40) |  | 1,547 (8.12) |  |
| Emilia\_Romagna\_Italy | 6,153 (6.11) | 1,820 (7.76) |  | 928 (4.87) |  |
| Vaud\_Switzerland | 2,501 (2.48) | 1,118 (4.76) |  | 931 (4.88) |  |
| Zagreb\_Croatia | 1,434 (1.42) | 309 (1.32) |  | 218 (1.14) |  |
| Antwerp\_Belgium | 4,303 (4.27) | 1,928 (8.22) |  | 1,078 (5.66) |  |
| Saxony\_Anhalt\_Germany | 2,962 (2.94) | 639 (2.72) |  | 418 (2.19) |  |
| Cork\_&\_Kerry\_Ireland | 2,196 (2.18) | 381 (1.62) |  | 710 (3.73) |  |
| Wales\_United\_Kingdom | 11,148 (11.07) | 3,523 (15.01) |  | 2,701 (14.17) |  |
| Norway\_Norway | 7,301 (7.25) | 1,316 (5.61) |  | 1,122 (5.89) |  |
| Wielkopolska\_Poland | 9,503 (9.44) | 1,837 (7.83) |  | 1,112 (5.83) |  |
| Poland\_Poland | 35,118 (34.87) | 5,652 (24.08) |  | 4,729 (24.81) |  |
| French\_West\_Indies-France | 428 (0.43) | 167 (0.71) |  | 256 (1.34) |  |
| Valencia\_Spain | 4,224 (4.19) | 1,329 (5.66) |  | 1,137 (5.97) |  |

**Table 3** Crude and AOR for the association between macrolides and each CA subgroup, using the primary exposure comparison (non-exposed), for macrolide signal CA and for CA previously associated with other antibiotics\*

|  | **Total** | **Exposed**  **n (%)** | **Non-Genetic Controls** | | | | **Genetic Controls** | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Crude model** | | **Adjusted modela** | | **Crude model** | | **Adjusted modela** | |
| **OR** | **95%CI** | **OR** | **95%CI** | **OR** | **95%CI** | **OR** | **95%CI** |
| **Macrolides\_J01FA** | | | | | | | | | | |
| **Non-Genetic Controls** | 23,467 | 72 (0.29) |  |  |  |  |  |  |  |  |
| **Genetic Controls** | 19,060 | 57 (0.28) |  |  |  |  |  |  |  |  |
| **All cases** | 100,702 | 304 (0.30) | 0.98 | [0.76, 1.27] | 0.99 | [0.76, 1.28] | 1.01 | [0.76, 1.34] | 1.04 | [0.77, 1.40] |
| **Literature signal for macrolides** | | | | | | | | | | |
| *Congenital heart defects* | 46,169 | 129 (0.28) | 0.91 | [0.68, 1.22] | 0.94 | [0.70, 1.26] | 0.93 | [0.68, 1.28] | 1.01 | [0.73, 1.41] |
| *Genital* | 12,450 | 40 (0.32) | 1.05 | [0.71, 1.54] | 0.96 | [0.65, 1.42] | 1.07 | [0.72 ,1.61] | 0.95 | [0.61, 1.46] |
| **Exploratory analyses of other antibiotic signal CA** | | |  | | | | | | | |
| Atrioventricular septal defect | 1,027 | 9 (0.88) | **2.87** | **[1.43, 5.76]** | **2.98** | **[1.48, 6.01]** | **2.95** | **[1.46, 5.97]** | **3.09** | **[1.48, 6.44]** |
| **Erythromycin\_J01FA01** | | | | | | | | | | |
| **Non-Genetic Controls** | 20345 | 28 (0.11) |  |  |  |  |  |  |  |  |
| **Genetic Controls** | 16761 | 18 (0.09) |  |  |  |  |  |  |  |  |
| **All cases** | 88270 | 119 (0.13) | 0.98 | [0.65, 1.48] | 1.00 | [0.66, 1.52] | 1.26 | [0.76, 2.06] | 1.30 | [0.78, 2.17] |
| **Literature signal for erythromycin** | | | | | | | | | | |
| *Congenital heart defects* | 39247 | 54 (0.14) | 1.00 | [0.63, 1.58] | 1.05 | [0.66, 1.67] | 1.28 | [0.75, 2.19] | 1.38 | [0.79, 2.40] |
| *Anencephalus and similar* | 1213 | 2 (0.16) | 1.20 | [0.29,5.04] | 1.02 | [0.24,4.32] | 1.54 | [0.36,6.63] | 1.12 | [0.25,5.05] |
| *Limb reduction* | 3121 | 4 (0.13) | 0.93 | [0.33, 2.66] | 1.27 | [0.44, 3.66] | 1.19 | [0.40, 3.53] | 1.31 | [0.42, 4.08] |
| **Exploratory analyses of other antibiotic signal CA** | | | | | | | | | | |
| Atrioventricular septal defect | 932 | 4 (0.43) | **3.13** | **[1.09, 8.94]** | **3.68** | **[1.28, 10.61]** | **4.01** | **[1.35, 11.87]** | **4.30** | **[1.40, 13.19]** |
| Diaphragmatic hernia | 1215 | 5 (0.41) | **3.00** | **[1.16, 7.78]** | **3.19** | **[1.22, 8.32]** | **3.84** | **[1.42, 10.37]** | **3.47** | **[1.25, 9.66]** |
| **Clarithromycin\_J01FA09¥** | | | | | | | | | | |
| **Non-Genetic Controls** | 11898 | 7 (0.03) |  |  |  |  |  |  |  |  |
| **Genetic Controls** | 10005 | 2 (0.01) |  |  |  |  |  |  |  |  |
| **All cases** | 60360 | 32 (0.05) | 0.90 | [0.40, 2.04] | 1.17 | [0.51, 2.65] | 2.65 | [0.64, 11.06] | 2.68 | [0.63, 11.34] |
| **Exploratory analyses of other antibiotic signal CA** | | | | | | | | | | |
| Oro-facial clefts | 6278 | 8 (0.13) | 2.17 | [0.79, 5.98] | **2.94** | **[1.04, 8.30]** | **6.38** | **[1.35, 30.06]** | **7.22** | **[1.47, 35.37]** |
| Cleft lip with or without palate | 3822 | 5 (0.13) | 2.23 | [0.71, 7.02] | 3.12 | [0.96, 10.16] | **6.55** | **[1.27, 33.78]** | **7.43** | **[1.35, 40.82]** |
| Cleft palate | 2456 | 3 (0.12) | 2.08 | [0.54, 8.04] | 2.45 | [0.62, 9.75] | **6.12** | **[1.02, 36.63]** | **6.81** | **[1.11, 41.74]** |
| **Azithromycin\_J01FA10** | | | | | | | | | | |
| **Non-Genetic Controls** | 13325 | 17 (0.07) |  |  |  |  |  |  |  |  |
| **Genetic Controls** | 11154 | 20 (0.10) |  |  |  |  |  |  |  |  |
| **All cases** | 64295 | 79 (0.12) | 0.96 | [0.57,1.63] | 1.14 | [0.67, 1.94] | 0.68 | [0.42, 1.12] | 0.85 | [0.50, 1.43] |
| **Literature signal for azithromycin** | | | | | | | | | | |
| *Congenital heart defects* | 28,735 | 27 (0.09) | 0.74 | [0.40,1.35] | 0.90 | [0.49,1.65] | 0.52 | [0.29,0.93] | 0.66 | [0.35,1.23] |
| *Oro-facial clefts* | 6,596 | 6 (0.09) | 0.71 | [0.28, 1.81] | 0.89 | [0.35, 2.28] | 0.51 | [0.20, 1.26] | 0.54 | [0.21, 1.40] |
| **Exploratory analyses of other antibiotic signal CA** | | | | | | | | | | |
| Atrioventricular septal defect | 657 | 3 (0.46) | **3.59** | **[1.05, 12.28]** | **4.50** | **[1.30, 15.58]** | 2.55 | [0.76, 8.62] | 2.74 | [0.78, 9.63] |
| Syndactyly | 2,211 | 8 (0.36) | **2.84** | **[1.23, 6.59]** | **3.80** | **[1.62, 8.94]** | 2.02 | [0.89, 4.60] | 2.18 | [0.92, 5.21] |
| **Clindamycin\_J01FF01** | | | | | | | | | | |
| **Non-Genetic Controls** | 13325 | 4 (0.02) |  |  |  |  |  |  |  |  |
| **Genetic Controls** | 11154 | 7 (0.03) |  |  |  |  |  |  |  |  |
| **All cases** | 67018 | 28 (0.04) | 1.38 | [0.48, 3.94] | 1.40 | [0.49, 4.01] | 0.63 | [0.28, 1.44] | 0.72 | [0.30, 1.72] |
| **Exploratory analyses of other antibiotic signal CA** | | | | | | | | | | |
| Hydrocephalus | 1,656 | 3 (0.18) | **6.00** | **[1.34, 26.82]** | **6.63** | **[1.46, 30.18]** | 2.74 | [0.71, 10.61] | 4.1 | [0.97, 17.32] |

\* Signal CA associated with other antibiotics – only statistically significant results with at least three exposed cases shown, Other results in Supplementary Tables S3-9; a Adjusted for year of birth, EUROCAT Registry and maternal age ; ¥ Showed elevated risk of atrioventricular septal defect based on 2 exposed cases **(AOR 6.85, CI: 1.41 – 33.32)**; Original signals are in italics.; Anophthalmia had increased Odds ratios, but this was based on one or two exposed cases (See Supplementary Table S4-9); Numbers in the comparison groups vary because for each specific antibiotic, registries without any exposures were excluded; Data for Spiramycin - J01FA02 presented in Supplementary Table S6.

Original Sample

(170,062)

SE Ireland, Reunion-France, & Ukraine registries **(11,245)**

TOPFA (Emilia Romagna Registry) **(2,444)**

Isolated hip dislocation only **(4,243)**

Pregestational diabetes/hypoglycaemics, Epilepsy/anti-epileptics **(2,538)**

Antibiotic exposure of unknown timing **(3,655) ¥**

**Exclusions (24,126)**

Final Sample

(145,936)

**Figure 1**: Sample size flow chart

*****One baby with isolated sequence of unspecified nature was also excluded*

***¥*** *(90% of registrations with unknown timing were from three registries (Saxony\_Anhalt\_Germany, Poland, and Wielkopolska\_Poland)*

Registrations with genetic syndromes

(8,383)

**All Congenital Anomalies (CA)**

**(Total Sample: 145,936 registrations)**

**Non-Genetic Controls(23,467)**

**All other EUROCAT subgroups**

**Other CA subgroupsIncluding:** Congenital Heart Defect subcategoriesOrofacial Cleft subcategoriesGenital subcategories

**Figure 2**: Classification of EUROCAT CA subgroups according to their status as signals in previous studies (up to 2016) of antibiotics and macrolides, used in designation of case and control groups. All malformations shown are EUROCAT subgroups defined in EUROCAT Guide 1.4.31

**CA subgroups associated with macrolides in previous literature(Macrolide Signals)**AnencephalyCongenital Heart Defects (combined)Orofacial Clefts (combined)Genital (combined)Limb reduction

**CASES**

**(100,702)**

Nervous System + subcategories

Anopthalmos (Eye)

C. Glaucoma (Eye)

Ear, Face and Neck + subcategories

Congenital Heart Defects + subcategories

Choanal Atresia (Respiratory)

Orofacial Clefts + subcategories

Oesophageal atresia +/- fistula (Digestive)

Atresia/stenosis small intestine (Digestive)

Diaphragmatic Hernia (Digestive)

Gastroschisis (Abdominal Wall)

Genital + subcategories

Limb + subcategories

**Genetic Controls**

**(19,060)**

**Registrations with anomalies related to cases** **(2,707)**

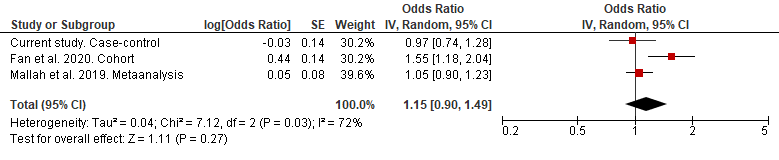
* Other eye (1,338)
* Duodenal atresia or stenosis (322)
* Anorectal atresia or stenosis (934)
* Atresia of bile ducts (113)

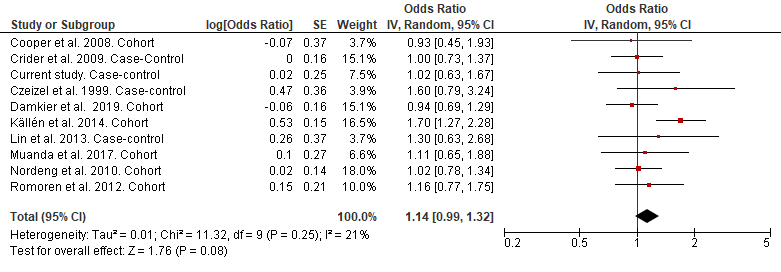
CONTROLS(36,851)

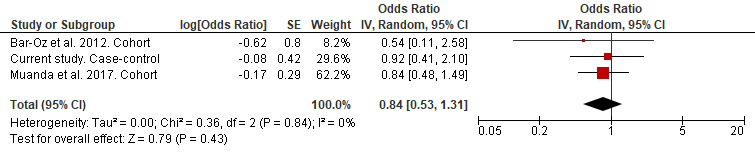
Registrations with CA associated with antibiotics in literature, including those with genetic syndromes

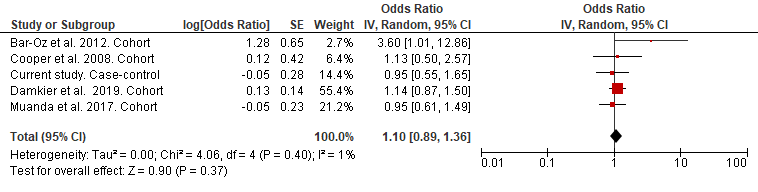
(109,085)

1. **Azithromycin**
2. **Clarithromycin**
3. **Macrolides**
4. **Erythromycin**









**Figure 3 A-D** Meta-analysis and forest plot for the association between CHD and first trimester use of macrolides, erythromycin, clarithromycin and azithromycin.