Prenatal exposure to antibiotics and wheezing in infancy: a birth cohort study

Maja Popovic[1,2], Franca Rusconi[3], Daniela Zugna[1,2], Claudia Galassi[2,4], Franco Merletti[1,2,4], Enrica Migliore[1,2,4], Morena Trevisan[2,4], Tiziana Nannelli[5], Luigi Gagliardi[6] and Lorenzo Richiardi[1,2,4]

Affiliations:
[1]Dept of Medical Sciences, University of Turin, Turin, Italy.
[2]CPO Piemonte, Turin, Italy.
[3]Unit of Epidemiology, "Anna Meyer" Children's University Hospital, Florence, Italy.
[4]AOU Città della Salute e della Scienza, Turin, Italy.
[5]Dept of Clinical and Experimental Medicine, University of Florence, Florence, Italy.
[6]Dept of Woman and Child Health, Paediatrics and Neonatology Division, Ospedale Versilia, Viareggio, Italy.

Correspondence: Maja Popovic, Dept of Medical Sciences, University of Turin, Via Santena 7, 10126 Turin, Italy. E-mail: maja_popovic@hotmail.com

ABSTRACT   The role of prenatal antibiotic exposure in the development of childhood wheezing is debated. We evaluated whether this association could potentially be explained by confounding factors.

Antibiotic use in the first and third trimester of pregnancy, wheezing in children aged ⩽18 months and confounding factors were assessed in singletons participating in the NINFEA (*Nascita e Infanzia: gli Effetti dell'Ambiente*) birth cohort (n=3530 for first-trimester exposure and n=3985 for third-trimester exposure).

There was no evidence of an association between antibiotic exposure in the first trimester of pregnancy and ever-wheezing (adjusted risk ratio (RR) 1.02, 95% CI 0.80–1.30) or recurrent wheezing (RR 0.99, 95% CI 0.54–1.82). For the third-trimester exposure, the crude RRs (95% CI) of ever-wheezing and recurrent wheezing were 1.34 (1.10–1.64) and 2.72 (1.80–4.11), respectively, which decreased to 1.12 (0.90–1.39) and 2.09 (1.32–3.29) after adjustment. The RRs of wheezing after genitourinary infections during pregnancy were increased independently of antibiotic treatment.

In conclusion, the association between prenatal antibiotic exposure and infant wheezing could be largely explained by confounding factors, in particular respiratory infections during pregnancy. An excess risk of wheezing after antibiotic exposure during the third trimester of pregnancy remains after adjustment.

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Confounders explain increased risk of infant wheeze after antibiotic exposure in the first but not third trimester http://ow.ly/Tu1U0

Introduction

There is growing evidence of the key role of the prenatal environment in the development of childhood wheezing [1, 2]. One of the well-known risk factors implicated in the development of wheezing and an associated diminished lung function is maternal smoking during pregnancy [2, 3]. The role of other prenatal exposures, including pregnancy complications or air pollution exposure, is less clear [4].

In recent years, several studies reported an increased risk of wheezing and asthma in infants and children after prenatal antibiotic exposure [5–18]. An imbalance of maternal vaginal and bowel bacterial flora caused by antibiotic treatment could lead to an abnormal early colonisation of infant gut and airway through vertical transmission during birth. It has been hypothesised that an altered composition of the infant's microbiota might cause a disruption of mucosal immune tolerance and lead to the development of T-helper type 2 mediated diseases [5, 19, 20].

Alternatively, the association between antibiotic use during pregnancy and childhood wheezing/asthma might stem from various confounding factors [21]. Potential confounders include risk factors for both maternal and childhood infections and wheezing such as maternal smoking and asthma, both associated with a greater risk of respiratory infections, and perinatal infections, *e.g.* chorionamnionitis, which might be treated with antibiotics [22].

Within the framework of a mother–child cohort study, we evaluated the association between antibiotic use during pregnancy and the risk of wheezing up to the age of 18 months in children. We aimed in particular at investigating to what extent these associations might be explained by confounding factors.

Methods

Study population

NINFEA (*Nascita e Infanzia: gli Effetti dell'Ambiente*; Birth and Childhood: Effects of the Environment) is an ongoing Italian internet-based birth cohort established in 2005 (www.progettoninfea.it) [23]. Members of the cohort are children of mothers who have access to the internet and enough knowledge of Italian to complete online questionnaires. Recruitment is conducted actively through obstetrics clinics, and passively *via* the internet and the media [24]. ~75% of participants are recruited actively, 20% passively and 5% comprise both modes. Participants complete the baseline questionnaire at any time during pregnancy and another four questionnaires at 6 and 18 months after delivery and when children turn 4 and 7 years of age.

We used the 2015.03 version of the NINFEA database and the information from the first three questionnaires. The study population consisted of 4421 singletons with information on occurrence of wheezing. Analyses regarding the first trimester exposure were restricted to 3812 children as we excluded 609 children born to mothers who completed the first questionnaire before the end of the first trimester of pregnancy. The NINFEA response rate at the 18-month questionnaire is 84%.

The study was approved by the ethical committee of the San Giovanni Battista Hospital and CTO/CRF/ Maria Adelaide Hospital (Turin, Italy) (approval N.0048362 and following amendments).

Exposure variables

Antibiotic exposure in the first trimester was assessed using the questionnaire completed after the end of the first trimester of pregnancy, while third-trimester exposure was assessed using the questionnaire completed 6 months after delivery. Second-trimester exposure was not evaluated in this study, as such analysis would have required an exclusion of all participants who completed the first questionnaire before the end of the second trimester, leading to a great reduction of the sample size. Duration of antibiotic use was also analysed. Questionnaires included additional information on any medication used during pregnancy to treat a number of chronic conditions, listed in a pre-specified checklist, from which we selected those that could represent an indication for antibiotic use (namely, genitourinary infections, including recurrent urinary infections, *Chlamydia* infections and kidney stones). Three categories were identified: 1) females with at least one of the three diseases not treated with antibiotics; 2) females with at least one of the diseases treated with antibiotics; and 3) females without any of the three diseases.

The exact wording of the questions used in the study is reported in online supplementary table S1.

Outcome variables

Ever-wheezing was defined as at least one episode of wheezing or whistling in the chest in the first 6 months or between 6 and 18 months of life, while recurrent wheezing was defined as wheezing or whistling in the chest reported both in the first 6 months and between 6 and 18 months of life. The exact wording of the questions used in the study is reported in online supplementary table S2.

TABLE 1 Main characteristics of the study population

	First trimester antibiotics		Third trimester antibiotics	
	No	Yes	No	Yes
Subjects	3301 (93.5)	229 (6.5)	3729 (93.6)	256 (6.4)
Maternal educational level				
University degree or higher	2031 (61.5)	150 (65.5)	2292 (61.5)	165 (64.5)
Secondary school	1132 (34.3)	69 (30.1)	1271 (34.1)	82 (32.0)
Primary school or less	138 (4.2)	10 (4.4)	166 (4.4)	9 (3.5)
Maternal age at childbirth years				
<30	654 (19.8)	44 (19.2)	727 (19.5)	43 (16.8)
30–34	1462 (44.3)	86 (37.5)	1634 (43.8)	110 (43.0)
35–39	977 (29.6)	70 (30.6)	1113 (29.9)	86 (33.6)
⩾40	208 (6.3)	29 (12.7)	255 (6.8)	17 (6.6)
Maternal asthma/asthmatic bronchitis				
Yes	247 (7.5)	20 (8.7)	276 (7.4)	25 (9.8)
No	3054 (92.5)	209 (91.3)	3453 (92.6)	231 (90.2)
Siblings				
Yes	975 (29.5)	95 (41.5)	1200 (32.2)	102 (39.8)
No	2326 (70.5)	134 (58.5)	2529 (67.8)	154 (60.2)
Maternal smoking in pregnancy				
Yes	257 (7.8)	20 (8.7)	289 (7.7)	20 (7.8)
No	3044 (92.2)	209 (91.3)	3440 (92.3)	236 (92.2)
Maternal paracetamol use in pregnancy				
Yes	1752 (53.1)	148 (64.6)	1989 (53.3)	174 (68.0)
No	1549 (46.9)	81 (35.4)	1740 (46.7)	82 (32.0)
Maternal respiratory infections in pregnancy				
Otitis and/or sinusitis and/or throat infections				
Yes	278 (8.4)	60 (26.2)	201 (5.4)	66 (25.8)
No	3023 (91.6)	169 (73.8)	3528 (94.6)	190 (74.2)
Bronchitis and/or flu				
Yes	175 (5.3)	33 (14.4)	153 (4.1)	48 (18.7)
No	3126 (94.7)	196 (85.6)	3576 (95.9)	208 (81.3)
Common cold				
Yes	621 (18.8)	48 (21.0)	463 (12.4)	67 (26.2)
No	3680 (81.2)	181 (79.0)	3266 (87.6)	189 (73.8)
Other maternal infections in pregnancy				
Urinary infections and/or urinary burning				
Yes	122 (3.7)	46 (20.1)	202 (5.4)	70 (27.3)
No	3179 (96.3)	183 (79.9)	3527 (94.6)	186 (72.7)
Vaginal infections				
Yes	194 (5.9)	62 (27.1)	353 (9.5)	80 (31.2)
No	3107 (94.1)	167 (72.9)	3373 (90.5)	176 (68.8)
Fever >38°C				
Yes	90 (2.7)	22 (9.6)	58 (1.6)	29 (11.3)
No	3211 (97.3)	207 (90.4)	3671 (98.4)	227 (88.7)
Mode of delivery				
Vaginal	2254 (74.3)	136 (65.4)	2548 (74.0)	159 (67.7)
Caesarean section	779 (25.7)	72 (34.6)	895 (26.0)	76 (32.3)
Wheezing up to 6 months				
Yes	220 (7.1)	22 (10.6)	268 (7.3)	34 (13.4)
No	2887 (92.9)	185 (89.4)	3410 (92.7)	219 (86.6)
Ever-wheezing up to 18 months				
Yes	690 (20.9)	60 (26.2)	803 (21.5)	74 (28.9)
No	2611 (79.1)	169 (73.8)	2926 (78.5)	182 (71.1)
Recurrent wheezing				
Yes	118 (3.8)	12 (5.7)	134 (3.8)	24 (10.4)
No	2989 (96.2)	200 (94.3)	3371 (96.2)	207 (89.6)

Data are presented as n (%).

In the 18-month questionnaire mothers were additionally asked to report if a paediatrician had ascertained wheezing episodes occurring between 6 and 18 months of age; doctor confirmation was reported in 89.8% of cases.

Confounding factors

Three sets of potential confounders were pre-selected: 1) core confounders, including maternal age and educational level at delivery, maternal smoking and paracetamol use during pregnancy, number of siblings of the child and maternal history of asthma; 2) respiratory infections during pregnancy including otitis, sinusitis, throat infections, bronchitis, flu and common cold; and 3) other infections during pregnancy that may represent an indication for antibiotic use, including vaginal and urinary infections as well as episodes of fever >38°C as a nonspecific indicator for infectious diseases. The confounding factors were coded as shown in table 1.

Statistical analyses

We used a complete case analysis approach which implied an exclusion of <10% of the subjects. The analyses included 3530 and 3319 subjects for the first-trimester exposure, and 3985 and 3736 subjects for the third-trimester exposure for ever-wheezing and recurrent wheezing, respectively. Crude and adjusted risk ratios (RR) with 95% confidence intervals were estimated using a Poisson regression model [25]. Since mothers could participate in the NINFEA cohort with more than one pregnancy we estimated robust variance using clustered sandwich estimators to allow for intragroup correlation. The amount of confounding by each confounders' set, as well as by the single factors, was quantified by the change-in-estimate criterion [26]. As we had no information on the antibiotic spectrum used, we performed an additional analysis by excluding mothers who took antibiotics to treat genitourinary infections (n=21 for the first trimester and n=51 for the third trimester) and compared the remaining mothers with those unexposed. We further restricted the analyses to mothers who used antibiotics for ⩾3 days with the purpose of reducing potential exposure misclassification. The presence of collinearity between antibiotic exposure and infections during pregnancy was checked by looking at the variance inflation factors for the final models [27].

Females undergoing Caesarean sections are typically pretreated with antibiotics [28], but are either unaware of it or simply underreport this exposure. As Caesarean sections might be associated with wheezing/asthma in childhood [29], in order to reduce possible misclassification we conducted a sensitivity analysis restricted to the vaginally delivered children (n=2707 for ever-wheezing and n=2536 for recurrent wheezing).

Finally, in order to evaluate confounding by indication we compared children of mothers with genitourinary infections who were treated with antibiotics with untreated ones. For completeness, we also estimated the association between genitourinary infections (regardless of antibiotic treatment) and infant wheezing.

All analyses were performed using Stata 13 (Stata Corporation, College Station, TX, USA).

Results

Main characteristics of the study population are presented in table 1. The prevalence of antibiotic use was 6.5% and 6.4% in the first and third trimesters, respectively, with an overlap of 18.5% between these two

TABLE 2 Associations between prenatal exposure to antibiotics (users *versus* nonusers) and wheezing outcomes in children aged ⩽18 months

Outcomes	Cases	Exposed cases	RR$_{crude}$	RR$_{adj1}$[#]	RR$_{adj2}$[¶]	RR$_{adj3}$[+]
First trimester						
Ever-wheezing	750	60 (8.0)	1.25 (1.00–1.57)	1.14 (0.91–1.43)	1.07 (0.85–1.35)	1.02 (0.80–1.30)
Recurrent wheezing	130	12 (5.7)	1.49 (0.84–2.66)	1.25 (0.70–2.24)	1.09 (0.60–1.98)	0.99 (0.54–1.82)
Third trimester						
Ever-wheezing	877	74 (8.4)	1.34 (1.10–1.64)	1.25 (1.02–1.52)	1.18 (0.95–1.46)	1.12 (0.90–1.39)
Recurrent wheezing	158	24 (15.2)	2.72 (1.80–4.11)	2.36 (1.57–3.54)	2.25 (1.44–3.53)	2.09 (1.32–3.29)

Data are presented as n, n (%) or risk ratio (RR) (95% CI). RR$_{adj}$: adjusted risk ratio. [#]: adjusted for maternal educational level, maternal age at birth, smoking in pregnancy, siblings, paracetamol use in pregnancy and maternal asthma or asthmatic bronchitis; [¶]: adjusted as RR$_{adj1}$ and for maternal respiratory infections (bronchitis, flu, throat infections, otitis, sinusitis and common cold); [+]: adjusted as RR$_{adj2}$ and for other maternal infections (urinary tract infections and vaginal infections) and fever >38°C.

exposures. Paracetamol use during pregnancy, Caesarean delivery and presence of siblings of the index child were more frequent among the exposed subjects (both trimesters p<0.03), as well as infections and fever >38°C (both trimesters p<0.001). Mothers exposed in the first trimester were older compared with the unexposed subjects (p=0.002). Children exposed in the third trimester suffered more often from both ever-wheezing and recurrent wheezing than those unexposed (p=0.028 and p<0.001, respectively).

The associations between prenatal antibiotic exposure and infant wheezing are reported in table 2. All crude estimates gradually attenuated after adjustment for each set of confounding factors. Only the association between recurrent wheezing and antibiotic exposure during the third trimester of pregnancy remained after adjustment (RR 2.09; 95% CI 1.32–3.29). In the third trimester, maternal respiratory infections were the strongest confounder of the association between prenatal antibiotic exposure and infant wheezing, and together with the core confounders had a strong confounding role in the first trimester (table 3). More precisely, maternal otitis, sinusitis and throat infections among respiratory infections, urinary infections among the other infections during pregnancy and presence of siblings (especially for the first-trimester exposure) among the core confounders had the strongest confounding effects on the associational estimates (online supplementary table S3). In addition, for all the associations between antibiotic use and infant wheezing the main confounding factors were positive, with the exception of fever which was a negative confounder for the associations involving the third-trimester exposure. All variance inflation factor values were below five, indicating a stable model with no evidence of multicollinearity problems.

Restricting the analyses to mothers treated with antibiotics other than those for genitourinary infections did not alter the results (data not shown).

In the sensitivity analysis intended to reduce potential exposure misclassification, restriction to mothers who used antibiotics during the first trimester for ⩾3 days yielded fully adjusted RRs (95% CI) of 1.01 (0.78–1.30) for ever-wheezing and 1.03 (0.54–1.95) for recurrent wheezing. In the same analysis for the third trimester exposure, fully adjusted RRs (95% CI) of ever-wheezing and recurrent wheezing were 1.06 (0.84–1.34) and 1.88 (1.16–3.05), respectively.

The analyses on the third-trimester antibiotics exposure restricted to children delivered vaginally yielded fully adjusted RRs (95% CI) of 1.09 (0.81–1.47) for ever-wheezing and 1.72 (0.88–3.34) for recurrent wheezing.

We found no evidence of an increased risk of wheezing among the children of mothers who used antibiotics to treat genitourinary infections compared with the children of mothers who did not use antibiotics to treat them (table 4). However, the confidence intervals are wide due to the substantially smaller sample size of this subgroup of the cohort. When all the mothers with genitourinary infections during pregnancy were compared with those without infections, we found, for the first trimester, adjusted RRs (95% CI) of 1.33 (0.97–1.81) for ever-wheezing and 2.43 (1.29–4.59) for recurrent wheezing, and for the third trimester, adjusted RRs (95% CI) of 1.34 (1.10–1.62) for ever-wheezing and 2.16 (1.39–3.34) for recurrent wheezing (online supplementary table S4).

Discussion

Our results show positive crude associations between prenatal antibiotic exposure and infant wheezing, but the estimates decrease and often annul after adjustment, particularly for maternal infections during pregnancy. An increased risk of recurrent wheezing after antibiotic exposure in the third trimester remained

TABLE 3 Change-in-estimate of the effect of antibiotic use on wheezing outcomes in the first and third trimesters of pregnancy

Confounding factors	Ever-wheezing	Recurrent wheezing
First trimester		
Core confounders *versus* crude model	−8.7	−16.0
Respiratory infections *versus* crude model	−8.0	−15.8
Other infections *versus* crude model	−6.3	−13.6
Third trimester		
Core confounders *versus* crude model	−6.9	−13.3
Respiratory infections *versus* crude model	−9.8	−13.5
Other infections *versus* crude model	−7.8	−8.5

Data are presented as %.

TABLE 4 Associations between antibiotic treatment for genitourinary infections (recurrent urinary tract infections or *Chlamydia* or kidney stones) and wheezing outcomes in children aged ⩽18 months in females who had genitourinary infections during pregnancy

	Cases	RR$_{crude}$	RR$_{adj1}$[#]	RR$_{adj2}$[¶]
First trimester				
Ever-wheezing[+]	98			
No antibiotics	18	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Antibiotics	11	1.05 (0.56–1.98)	0.90 (0.48–1.71)	0.95 (0.48–1.86)
Recurrent wheezing[§]	95			
No antibiotics	7	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Antibiotics	3	0.73 (0.20–2.68)	0.72 (0.19–2.74)	0.93 (0.18–4.72)
Third trimester				
Ever-wheezing[f]	266			
No antibiotics	50	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Antibiotics	28	1.06 (0.72–1.55)	1.06 (0.73–1.52)	1.05 (0.73–1.52)
Recurrent wheezing[##]	249			
No antibiotics	14	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
Antibiotics	7	0.95 (0.40–2.23)	1.00 (0.45–2.55)	1.06 (0.46–2.43)

Data are presented as n or risk ratio (RR) (95% CI). RR$_{adj}$: adjusted RR; ref.: reference. [#]: adjusted analysis for maternal educational level, maternal age at birth, smoking in pregnancy, siblings, paracetamol use in pregnancy and maternal asthma or asthmatic bronchitis; [¶]: adjusted analysis as RR$_{adj1}$ and for maternal respiratory infections (bronchitis, flu, throat infections, otitis, sinusitis and common cold) and fever >38°C; [+]: total subjects n=98; [§]: total subjects n=95; [f]: total subjects n=266; [##]: total subjects n=249.

even after adjustment for a number of confounding factors. The same pattern was found when only mothers who used antibiotics for ⩾3 days or those treated with antibiotics for infections other than genitourinary ones were considered as exposed. Genitourinary infections in pregnancy were associated with an increased risk of infant wheezing, regardless of whether or not they had been treated with antibiotics.

A number of studies [5–18] assessed the association between prenatal antibiotic exposure and risk of wheezing and asthma in children. Most of them found a positive association, but the estimates were weak in some of the studies. STOKHOLM and colleagues [15, 16] found a dose-related association between maternal antibiotic use during pregnancy and the risk of asthma in the offspring. As the associations also existed for maternal antibiotic use before and after pregnancy, they suggested that antibiotic use in pregnancy might be a proxy for mothers' general susceptibility to infection. In a large population-based registry, ÖRTQVIST *et al.* [17] found a positive association between prenatal antibiotic exposure and asthma in the offspring, but these findings were not confirmed in a sibling analysis, indicating a confounding role of shared familial factors. Similarly, two meta-analyses attributed a positive association between antibiotic exposure in infancy and subsequent asthma to confounding factors [30, 31].

In our analyses we accounted for the most common infections during pregnancy, and we found that respiratory and urinary infections play the strongest confounding role. Nevertheless, the role of other confounders, such as presence of siblings, cannot be neglected. Furthermore, maternal educational level, a strong determinant of socioeconomic status and access to medicines, including self-medication, has been taken into account in the analysis. However, a persistent association between antibiotic exposure during the third trimester of pregnancy and infant wheezing existed even after controlling for multiple confounders. As Caesarean section is suggested to be associated with wheezing in childhood [29], this association could be to some extent explained by the mode of delivery, as the estimate slightly decreased after exclusion of children delivered by Caesarean section. However, a positive association, although attenuated and having large confidence intervals, remained in children delivered vaginally. The role of Caesarean section in the relationship between late pregnancy antibiotic exposure and the development of wheezing in childhood still needs to be elucidated.

Six previous studies analysed exposures for each trimester of pregnancy separately and three of them [7, 11, 18] found the strongest associations for exposure in late pregnancy, especially during the third trimester. In the other three studies [6, 15–17] associations did not depend on the period of exposure. The composition of the maternal commensal flora, which is influenced by antibiotic use in late pregnancy [32], may modify the maturation of the newborn immune system by altering normal microbial colonisation of the infant gut or airways [19]. These alterations of the immune system might lead to an atopic phenotype and an increased susceptibility to infections/wheezing.

In addition, we found an increased risk of infant wheezing after maternal genitourinary infections in pregnancy, regardless of antibiotic use, suggesting that these infections *per se* might be associated with wheezing in offspring. Consistently, in a previous cross-sectional study [8], antibiotics administered for maternal urinary infections were associated with transient early childhood wheezing, and an association between maternal urinary infections during pregnancy and childhood asthma has been found in two cohort studies [13, 33]. Abnormal colonisation of the genitourinary tract and ascending infections are possibly a proxy of chorioamnionitis [34], which in turn could play a role in modulating lung development [22].

Our study had some limitations. The NINFEA questionnaires do not include information on type of antibiotics administered, and analysing them all together yields an "average" effect of antibiotics, preventing us from estimating possibly diverse effects of different antibiotic classes, and of the mechanisms involved. Restriction of the exposed population to mothers who took antibiotics for infections other than genitourinary ones, assuming that most of them suffered from respiratory infections and hence used broad-spectrum antibiotics, did not change the results.

Our study is based on self-reported information, which may introduce misclassification. However, misclassification of the exposure was most probably driven by underreporting, rather than overreporting, which translates into high specificity and a reduced impact of nondifferential misclassification for an exposure with 6–7% prevalence [35]. Moreover, analyses restricted to mothers who used antibiotics for ⩾3 days and to those treated for genitourinary infections, designed to have a higher specificity and less potential for bias from nondifferential misclassification, did not alter the results.

We also relied on self-reported information when defining the outcome. Wheezing reported by mothers, although widely accepted in large epidemiological studies, might overestimate the presence of the condition, in particular ever-wheezing, as parents might label a single episode of noisy breathing as wheezing [36]. Recurrent wheezing is less affected by this source of bias and is more likely to identify a pathological condition [36].

As we studied children aged ⩽18 months we were unable to distinguish different wheezing phenotypes. Follow-up at older ages is therefore needed in order to clarify the role of prenatal antibiotics, if any, in the development of asthma and wheezing persisting into school age.

Another limitation is that the associational estimate between the third trimester exposure and recurrent wheezing might be affected by recall bias, as this exposure is assessed at the 6-month questionnaire together with wheezing in the first 6 months of life, which is a necessary component of the definition of recurrent wheezing. This could be relevant as we found an effect for the third trimester exposure only.

As in many other cohort studies, members of the NINFEA cohort mainly originate from a selected population with high education and socioeconomic status. However, it has been shown extensively that this baseline selection does not imply biased associational estimates in cohort studies [37, 38]. Furthermore, in the NINFEA cohort, the distribution of the exposure and outcomes did not differ between different levels of education.

In conclusion, the associations between prenatal antibiotic exposure and early childhood wheezing are largely explained by confounding factors, in particular maternal infections during pregnancy. Although multiple adjustments and the exclusion of children delivered by Caesarean section attenuated the magnitude of the association, the risk of recurrent wheezing after maternal antibiotic use in the third trimester remained increased. Our data also suggest a positive association between genitourinary infections during pregnancy and infant wheezing, independently of antibiotic use. These results need replication as our analysis was not originally designed to address this specific association.

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