Early-life antibiotic use is associated with wheezing among children with high atopic risk: a prospective European study

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Abstract

Background: Little is known about the relationship between antibiotic use and asthma in the children with a higher risk of allergic sensitization. We examine the association between the use of specific therapeutic antibiotics in the first year of life and development of wheezing by 36 months among children with a higher risk of allergic sensitization. Methods: A multi-center prospective cohort study was conducted among children at high risk for allergic sensitization. A validated questionnaire was used to prospectively collect information on antibiotic use and potential risk factors for wheezing from parents or guardians of 606 children from three European countries at 6, 12, 24 and 36 months of age. Multivariate linear and logistic regression models were used to adjust for potential confounders and effect modifiers and to estimate the association of antibiotic use with the development of early childhood wheezing. Results: Of the antibiotics assessed, only macrolide use in the first year of life was associated with increasing risk for wheezing by 36 months, after adjusting for gender, socioeconomic status, breast feeding >6 months, tobacco smoke exposure, family history of asthma, and respiratory infection (RR = 1.09; 95% CI 1.05–1.13). To avoid a bias by indication, we analyzed children with and without respiratory infection separately. Similar associations were observed for macrolides use in children who had no respiratory infection. Conclusions: In European children with a familial risk for allergic sensitization, we found a positive association between macrolide use in the first year of life and wheezing until 36 months old which was independent of the effect of respiratory infection.

Introduction

It has become clear within the past 20 years that much of the western world is experiencing an epidemic of childhood asthma and wheezing [1]. Several hypotheses have surfaced that partially explain the observed patterns of asthma prevalence throughout the world. One such postulation is known as the ‘‘old friends hypothesis’’ [2–4] which states that we are now exposed to different bacteria that do not have the same protective effect on our immune systems as the bacteria which coevolved with us. In other words, children are being raised in environments which are too clean – without sufficient microbial challenges to their developing immune systems. But what if a child is exposed sufficiently but not permitted to fully respond to the exposure challenge due to a pharmacologic intervention, such as an antibiotic medication? That question has fostered interest in early-life antibiotic exposures as risk factors for allergic illnesses [5]. For example, among high-risk children, Lapin et al. [6] found a positive association between early antibiotic exposure and asthma. Others have reported a dose-response relationship between antibiotic exposure and asthma [7]. However, there is still some uncertainty about the relationship between antibiotic use and subsequent asthma [8]. Within the past decade several studies have suggested that early-life exposure to antibiotics is associated with the etiology of childhood asthma [9,10]. Marra et al. [11] did a systematic review study and found that the pooled OR from retrospective studies was significantly stronger than the prospective studies (OR = 2.85; 95%CI 2.07–3.85 versus OR = 1.12; 0.88–1.42). However, previous studies have focused on clinical antibiotic exposure routes with the primary hypothesized mechanism being the interruption of the beneficial effects of infectious challenge [12]. Therefore, most of the previous studies assessed the effects of antibiotics in the presence of some type of bacterial infection. Hence, most of the previous epidemiological studies were confounded by some degree of infection, usually respiratory. Such a setting facilitates associations that indeed are due to prior infection rather than the medication to treat that infection (indication bias). On the contrary, antibiotics

Keywords

Antibiotic use, children, prospective cohort, wheezing

History

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themselves may have a direct influence on the development of asthma. If this truly is the case, then early life exposure to different antibiotic classes should be associated with different effects on the prevalence of pediatric asthma. This hypothesis was validated in an animal model where Russell et al. [13] found that administering vancomycin to mice early in life shifts resident gut flora and enhances future susceptibility to allergic asthma. This effect was not observed in mice given another antibiotic, streptomycin, nor when either antibiotic was administered to adult mice [13].

We hypothesized that the association of asthma with early life antibiotic exposure varied across antibiotic class and was independent of infection. To test this hypothesis, we analyzed data from a prospective birth cohort study of a population of children selected from different regions across Europe which had prospectively collected data on both asthma symptoms and antibiotic use.

**Methods**

**Study population and follow-up**

Details on the methods used to develop the Study of Prevention of Asthma in Children in Europe (SPACE) birth cohort have been reported elsewhere [14–18]. Briefly, this birth cohort was established in 2000, enrolling newborns from hospitals in England, Germany and Austria. Questionnaires were used to ascertain the child’s allergic and respiratory symptoms and antibiotic use at 6, 12, 24 and 36 months of age. All questionnaires were well validated and from the International Study of Allergy and Asthma in Children (ISAAC) [19–23].

**Inclusion and exclusion criteria**

We included newborns who had (1) parental plans to reside in the defined area, a 50 km radius around the medical center (urban and rural mix); and either (2) a family history of allergic sensitization (asthma, hay fever or eczema in either a parent or sibling); or (3) a positive skin prick test (SPT) or IgE in at least one parent indicative of atopy [15–18]. Exclusion criteria were birth weight below 2500 g and admission to a neonatal intensive care unit for longer than 7 days [18].

**Exposure measurement – antibiotic use**

An annual antibiotic use diary was provided to the parents for each year of study so that they could prospectively record the history of antibiotics prescribed for their child. Information was then prospectively collected regarding prescribed antibiotics in the past 12 months by self-reported questionnaire at 6, 12, 24 and 36 months of age. However, the data collected in the 12-month questionnaire were used exclusively as the exposure measure in this analysis. Prescriptions renewed within 7 days were considered as a single course. Antibiotics were categorized into six classes: Cephalosporin, Ampicillin, Macrolides, Penicillin, Trimethoprim and wide spectrum antibiotics.

**Outcomes measurement – wheezing**

Wheezing symptoms within the previous 12 months were our primary outcome measure, and was queried by the question “Has your child had wheezing or whistling in the chest in the last 12 months?” Questionnaires were completed at 6, 12, 24 and 36 months of age prospectively.

**Data analysis**

Chi-square tests were used to compare participants’ demographic characteristics by antibiotic use class. We had four repeated measurement periods. Repeated measurements and GEE (generalized estimating equation) were used to adjust for potential confounders and effect modifiers of the estimated Relative Risks (RR) for antibiotic use with the development of wheezing and 95% confidence intervals (CI). Time was considered as a repeated measurement and country was considered as a nested factor. For the within-subject association, we assumed a first-order autoregressive covariance structure. Country was further considered as a random effect. Potential confounders which were considered included gender, socioeconomic status, breast feeding greater than six months, infant second-hand passive smoke exposure, and respiratory infection. Alpha was set to the 0.05 level (two-sided). SAS 9.3 (SAS Institute, INC, Cary, NC) was used for all statistical analyses. Ethics approval was obtained from the local Ethics Committee in each study center. The study complied with the Helsinki Declaration.

**Results**

We enrolled 696 infants from three counties in a birth cohort with 670 (96.3%) followed up to 36 months of age. We calculated the sample size with the alpha of 0.05 (two sides) and power of 0.80 (n = 66). There were 606 (87.1%) out of 670 participants who responded to the first year of life questionnaire and then followed for 36 months. Mothers primarily completed these questionnaires (97.5%). We found that 76.6% of parents claimed that their child did not use any antibiotics within the first 36 months while 15.4% used at least one course of antibiotics. There was a higher prevalence of wheezing in children 12 months of age with a respiratory infection than those without a respiratory infection (Table 1). Children from England or those with a higher education level mother had a higher prevalence of wheezing by 36 months of age (Table 1). The prevalence of the wheezing within the previous 12 months among the children at 12, 24 and 36 months were 3.38%, 3.39% and 4.74%, respectively.

Table 2 shows that after adjusting for maternal education level, birth order, and country in a repeated measures analysis, the use of macrolides (but no other antibiotics) in the first year of life was significantly associated with increased risk for wheezing by 36 months of age (RR = 1.09; 95%CI 1.05–1.13). Further adjustment for breastfeeding for more than 6 months provided similar results (data not shown). After stratifying by lower respiratory-tract infection (LRI), similar associations were observed for macrolides use among the children without LRI (RR = 1.07; 95%CI 1.04–1.10), and with LRI (RR = 1.07; 95%CI 0.91–1.25). There was no association between the other antibiotics used and wheezing among the children with and without LRI (data not shown).
Early antibiotics use and childhood wheeze

Table 1. Characteristics of enrollees queried at 12 months from three European countries and wheezing by 36 months of age.

| Variables                      | n    | Wheezing (%) | p Value |
|--------------------------------|------|--------------|---------|
| Gender                         |      |              |         |
| Male                           | 277  | 5.05         | 0.72    |
| Female                         | 272  | 4.41         |         |
| Breastfeeding ≥6 Months        |      |              |         |
| Yes                            | 59   | 1.69         | 0.28    |
| No                             | 426  | 4.76         |         |
| Passive smoking (Parents are current smoker) |      |              |         |
| Yes                            | 119  | 5.88         | 0.51    |
| No                             | 430  | 4.41         |         |
| Paternal history of asthma     |      |              |         |
| 1                              | 186  | 3.76         | 0.56    |
| 2                              | 181  | 6.08         |         |
| 3                              | 182  | 4.40         |         |
| Cephalosporine                 |      |              |         |
| Yes                            | 50   | 4.88         |         |
| No                             | 494  | 4.45         |         |
| Ampicillin                     |      |              |         |
| Yes                            | 47   | 8.51         | 0.15    |
| No                             | 497  | 4.02         |         |
| Macrolides                     |      |              |         |
| Yes                            | 29   | 10.34        | 0.11    |
| No                             | 515  | 4.08         |         |
| Penicillin                     |      |              |         |
| Yes                            | 15   | 6.67         | 0.66    |
| No                             | 529  | 4.35         |         |
| Trimethoprim                   |      |              |         |
| Yes                            | 3    | 0            | 0.71    |
| No                             | 541  | 4.43         |         |
| Wide spectrum                  |      |              |         |
| Yes                            | 25   | 8            | 0.37    |
| No                             | 519  | 4.23         |         |
| Lower respiratory infection (Previous 1 year) |      |              |         |
| Yes                            | 71   | 15.49        | <0.001  |
| No                             | 473  | 2.74         |         |
| Other infections               |      |              |         |
| Yes                            | 55   | 1.81         | 0.32    |
| No                             | 489  | 4.71         |         |
| Mother education               |      |              |         |
| High                           | 132  | 1.52         | <0.001  |
| Medium                         | 293  | 4.10         |         |
| Normal                         | 124  | 9.68         |         |
| Country                        |      |              |         |
| Austria                        | 285  | 2.81         | <0.001  |
| England                        | 135  | 13.33        |         |
| Germany                        | 129  | 0.01         |         |

Table 2. Adjusted relative risks with 95% confidence intervals associated with exposure to antibiotic in the first year of life and wheezing by 36 months of age as assessed at 6, 12, 24, and 36 months in a repeated measures analysis.

| Variables | ALL | With LRI | Without LRI |
|-----------|-----|----------|-------------|
| Cephalosporine | 1.02 (1.00–1.05) | 1.00 (0.86–1.15) | 1.00 (0.98–1.01) |
| Ampicillin    | 0.99 (0.94–1.03) | 0.89 (0.77–1.04) | 0.99 (0.95–1.02) |
| Macrolides    | 1.09 (1.05–1.13)* | 1.07 (0.91–1.25) | 1.07 (1.04–1.10)* |
| Penicillin    | 1.00 (0.94–1.05) | 1.03 (0.77–1.36) | 1.02 (0.99–1.05) |
| Trimethoprim  | 0.98 (0.88–1.09) | 0.94 (0.53–1.68) | 1.00 (0.93–1.07) |
| Wide spectrum | 1.02 (0.98–1.06) | 1.04 (0.86–1.27) | 0.99 (0.97–1.02) |

*p<0.05; LRI: Lower respiratory infection. Adjusted for the fixed effects of birth order, paternal and maternal asthma, and mother education level and a country random effect

Discussion

Except for macrolides, we found that antibiotic use in the first year of life was unassociated with parent-reported wheezing symptoms by 36 months of life after adjustment for other asthma risk factors; such as birth order, paternal and maternal asthma, mother education level, and country, and LRI. Further adjustment for breast feeding, smoking status of parents, and gender did not significantly improve model results (data not shown).

Our study is consistent with previous studies [9,24–26]. Foliaki et al. [26] found that antibiotic use in the first year was associated with asthma symptoms in a cohort study of 193 412 children from 29 countries (RR was 1.70 with 95%CI 1.60–1.80). It should be noted that they defined asthma symptoms as wheezing in the previous 12 months, as we did. Marra et al. [27] conducted a population-based study in Canada of 251 817 children and found a dose-response relationship between the number of the course of antibiotics in the first year of life and the development of asthma after 5 years of follow-up. They also reported that early life exposure to Macrolides was associated with asthma (RR = 1.11; 95%CI 1.06–1.17). Paul et al. [28] used the data from the US National Hospital Ambulatory Medical Care Survey and found that 48.8% of antibiotic prescriptions from 1998 to 2007 were for macrolides.

However, the association between antibiotic use in early life and asthma has not been shown in all previous studies. Celedon et al. [29] reported that antibiotic use in the first year of life was not associated with asthma that was initially diagnosed between the ages of 2 and 5 years of age in an urban US cohort. They could have under-estimated the risk because they did not have any information on parental history of asthma and allergies and other risk factors during childhood. Penders et al. [30] conducted a systematic review of antibiotic use and wheeze including 21 longitudinal studies and found that the pooled RR was 1.27 (95%CI 1.12–1.43) and the pooled RR attenuated to 1.12 (95%CI 0.98–1.26) after eliminating studies with possible reverse causation and confounding by indication (respiratory tract infections leading to antibiotic use may be the underlying cause triggering asthma symptom development). These results are very similar to our findings. However, Su et al. [31] followed a non-selected birth cohort of 424 children and found no relationship between antibiotic use and asthma after adjusting for potential confounding, such as 3-month breast feeding practice and gender.

Lower respiratory infection plays an important role in the study of the association between antibiotic use and asthma. The increased prevalence of pathogens in such cases, such as Haemophilus and Staphylococcus spp., may contribute to wheezing illnesses in this early age group [32]. Recent evidence from pediatric clinical practice also showed that appropriate diagnosis and treatment of childhood asthma can reduce excessive antibiotic usage [33]. On the other hand, the use of the antibiotics may, also, be a consequence of an increased occurrence of respiratory infection in children with asthma. There is higher antibiotic consumption in children with asthma compared to those without asthma [34]. De Boeck et al. used a health insurance database that encompassed the records from 892 841 Belgian children seen in 1 year to examine antibiotic-prescribing practices. They reported that 36.62% of the children without an asthma diagnosis received an antibiotic while 73.50% children with...
an asthma medication did so [35]. Stallworth et al. [36] reported that pediatric asthmatic patients received significantly more antibiotic prescriptions than non-asthmatics for conditions caused by bacteria as well as for conditions more likely to be viral in origin. Protopathic bias is very common in similar studies due to the short follow-up period. Fortunately, the longer time of follow-up in our study may minimize potential protopathic bias.

There are several possible mechanisms which could explain how antibiotic use is associated with an increasing risk of asthma. One such explanation is that antibiotic use in infancy can cause changes in bowel microflora [37]. However, such changes are associated with various types of antibiotics and broad spectrum antibiotics could be more effective at reducing gut microflora. But many antibiotics have anti-inflammatory properties, also. An alternate potential biological mechanism is the antibiotic-related suppression of inflammatory responses in the course of treatment may later lead to increased immune response in Th2 shifted children or an impairment of Th1 immune responses in early childhood [38]. The regulation of the immune system depends on the balance between Th1 and Th2. Atopy develops when the balance deviates towards the Th2 cells. The Th2 cells predominate the immune system at birth. But environmental factors shift the balance of the immune system away from Th2 cells towards Th1 cells, which prevent the development of allergies [39]. However, this change does not fully occur in children with atopy [40].

Macrolides and some other antibiotics can suppress the production of proinflammatory cytokines, decrease mucus synthesis and promote inflammatory cell apoptosis in bronchial epithelium [41,42]. An anti-inflammatory mechanism had been reported that was related to allergic asthma based on reductions of various allergic responses via regulating small G proteins/MAP kinases/NF-kappa B in a mouse allergic asthma model [43]. Previous study indicated that macrolides may act by reducing pulmonary inflammation through reduction of neutrophil accumulation, resulting in reductions in the chemotactic gradient and cytokine production at the inflammatory sites in the lung or of reduced neutrophil adhesion molecules in the circulation [44]. This mechanism might suppress neutrophil oxidative and proteolytic products. Lymphocytes are important cellular components of bronchial inflammation [44]. Macrolides may act in part by inhibiting T-cell activation as an immunosuppressant [45]. Further study is needed to explain the mechanism by which macrolides are associated with early-childhood asthma.

Our study had several limitations. Despite the prospective data collection and antibiotic diaries, there may be potential recall bias because parents of children with wheezing may be more likely to remember their antibiotic use when they reported it for the 12 month questionnaire [26]. We found that 96 of the parents did not recall the type of antibiotic which their child was prescribed (36.2% of children reportedly treated with antibiotics). Furthermore, asthmatic parents of study participants may be more likely to pay attention to the medication of all their children, either with or without wheezing. The outcome in the present study is wheezing, not the physician diagnosis of asthma. However, nearly 30% of children have at least one episode of wheezing before their third birthday, and by 6 years the prevalence is almost 50% [46]. Some important potential confounders, such as maternal use of antibiotics before and during pregnancy [47], could not be adjusted for in our study. Finally, our study was performed on a relatively homogenous population. Some asthma-related genetic risk factors vary across different populations [48]. Further observational studies focusing on such different populations may help clarify this association between antibiotics use and asthma. Overall, these limitations do not explain the increased risks associated with early childhood macrolide antibiotic use.

There were several strengths to our study. First, the cohort consists of children with an atopic family history which may reduce recall bias. Risnes et al. [49] reported that family history modified the association between antibiotic use and asthma (P for interaction = 0.03) and the OR was 1.89 (95% CI 1.00–3.58) in children with no family history of asthma. We did not observe this modification effect in our study (P for interaction >0.05, data not shown), but our study was limited to families with a family history of atopy. Voor et al. [50] found antibiotic use is a risk factor for asthma in Estonia but not in Sweden; perhaps this is because broad-spectrum antibiotics were more likely prescribed in Estonia while penicillin in Sweden. We found no other study which contrasted the antibiotic risk factor for asthma across countries.

Second, we categorized antibiotics into six groups. Combining the groups may have masked the relationship of a single individual antibiotic if only one was truly associated with higher risk of asthma [31]. Studies have shown that the use of broad-spectrum cephalosporin can lead to marked disruptions of the intestinal microflora [51–53]. Jedrychowski et al. [38] pointed out that early childhood use of broad spectrum antibiotics but not other antibiotics was associated with an increased risk of developing asthma in 5-year-olds. Furthermore, Jedrychowski et al. [38] conducted a birth cohort with 310 Polish children and found that the overall use of antibiotics during early childhood was insignificantly associated with asthma (OR = 1.65, 95% CI: 0.93–2.93), although the crude OR was significant (OR = 2.14, 95% CI: 1.37–3.34). There have been several additional reports of asthma associated with clinical antibiotic exposures in the literature [30–35]. One such study found an extremely elevated prevalence of asthma in children with high exposures to clinical antibiotics in the first three years of life, especially in those with non-atopic asthma [30]. However, a similar study did not find such a relationship [36]. None of these clinical studies differentiated between antibiotic class, and were from different regions of the world. Therefore, divergence of the findings between these studies may be explained by their different antibiotic prescription patterns. In addition, none of these studies stratified antibiotic exposure for children with and without any concomitant respiratory infection.

Antibiotic use and misuse has ballooned in the past thirty years, as have the rates of childhood asthma. Antibiotics have been routinely used for a variety of illnesses, (such as the common cold) that are clearly viral and non-responsive to antibiotics. Furthermore, childhood middle ear infections, though very common, are usually treated idio pathically with
antibiotics on a trial and error bases. It is medically plausible that excessive exposure to antibiotics in early childhood may alter the developing inflammatory response to become sensitized to animal, plant, and fungal proteins. The clinical implications of our finding are that not all antibiotics used may be associated with asthma in children.

To our knowledge, this is the first longitudinal multi-center birth cohort study of antibiotics use and asthma among European children. Large prospective observational studies are warranted to confirm this observed association, especially in international populations. Longer longitudinal follow-up until the age of at least 6–8 years is also needed to further verify the development of asthma.

**Conclusion**

We found a positive association between macrolides use in the first years of life and later wheezing by age 3 years within European children with a familial risk for allergic disease. We recommend that physicians prescribe macrolide antibiotics responsibly.

**Declaration of interest**

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this article.

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Supplementary material available online