

REVIEW



The association between early life antibiotic use and allergic disease in young children: recent insights and their implications

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ABSTRACT

Introduction: Greater prescribing of antibiotics to infants has coincided with an epidemic of allergic disease. Through meta-analytic synthesis, accumulating evidence from prospective or database cohorts suggests a link between infant antibiotic treatment and the development of atopy. Stronger associations seen with multiple course and broad-spectrum antibiotic treatment add to biological plausibility. A major bias, confounding by indication, has been addressed in studies on antibiotic treatment of conditions which do not precede allergic disease.

Areas covered: Our review provides an up-to-date synthesis of the current literature on associations between infant antibiotic exposure and future allergic disease. We discuss methods that assist in reducing study bias and look at new insights from studies of the infant gut microbiome.

Expert commentary: Large-scale profiling of the gut microbiome provides a new tool for disentangling biases found in observational studies of infant antibiotic use. To date, microbial dysbiosis of the infant gut has been reported to predict allergic disease independent of antibiotic exposure up to 3 months after birth. However, these studies have not accounted for antibiotic treatment in later infancy. Continued study of the infant gut microbiome, mycobiome, or resistome will provide a closer link to antibiotic treatment or refute it as a cause of allergic disease.

ARTICLE HISTORY

Received 15 June 2018

Accepted 5 September 2018

KEYWORDS

Antibiotics; asthma; allergic disease; infants; microbiome; mycobiome

1. Trends in antibiotic use in infants

Antibiotics have been central to modern medicine since their introduction in the 1940s and their total global consumption has risen substantially over time [1]. Antibiotics that are commonly dispensed to children in early life include penicillins, cephalosporins, and macrolides. In the United States, efforts to reduce antibiotic prescribing have been ongoing and while overall usage rates declined during the 1990s [2] and from 2000 to 2010 [3], the prescribing rate of broad-spectrum antibiotics to children below and above 5 years has increased by 49% and 79%, respectively, in subsequent years [4]. In developed countries, the greater prescribing of antibiotics to young children has coincided with an epidemic of childhood asthma and allergic diseases [5]. Antibiotic use during infancy has been linked with later development of allergic diseases in childhood, although the published evidence is not entirely consistent; summary estimates from meta-analyses indicate a positive association [6–9], but a few individual studies have shown null [10–12] or even inverse associations [13,14]. Hence, the question of causality is yet to be answered.

Infections in children for which antibiotics are commonly prescribed are those of the respiratory tract such as acute otitis media (ear infection), sinusitis, pharyngitis and bronchitis, and of the urinary tract. Except in cases of penicillin allergy, clinical practice guidelines do not recommend broad-

spectrum macrolide and third-generation cephalosporin antibiotics as first-line treatment for common childhood respiratory tract infections [15–17]. Due to increasing microbial resistance and no apparent benefit, prophylactic antibiotic treatment of urinary tract infections in children is also not recommended [17]. Yet Vaz et al. [3] found that respiratory tract infections accounted for more than 75% of outpatient antibiotics prescriptions for children, of which otitis media was the most common diagnosis, especially in children between 3 and 24 months of age. The macrolides were the most commonly used antibiotic for otitis media. An increasing proportion of newborns and infants is also being treated in hospital with antibiotics for sepsis, lower respiratory tract infections, and pneumonia [18,19]. Versporten et al. [18] observed that broad-spectrum antibiotics, mainly third generation cephalosporins, were prescribed for children in Eastern Europe, Southern Europe, Asia, North America, and Latin America. More narrow-spectrum antibiotics like benzylpenicillin, amoxicillin, and gentamycin were commonly administered in other regions. Regional differences in the antibiotic treatment of hospitalized children are of concern and may be a sign of emerging antimicrobial resistance. Antibiotic stewardship programs and numerous campaigns to improve adherence to prescribing guidelines have been implemented over the last few decades to promote treatment with narrow-spectrum

antibiotics active against targeted pathogens. Despite their success, antibiotics remain overprescribed.

An overlooked source of infant exposure to antibiotics is their administration to the mother during birth. Common indications for the prophylactic use of antibiotics during delivery (intrapartum antimicrobial prophylaxis [IAP]) are to prevent early onset group B *Streptococcus* infection (GBS) in the newborn and wound infection in the women secondary to cesarean section (CS) delivery. At 40% of neonates, this combined use represents the main cause of antibiotic exposure during the perinatal period [20]. Mothers colonized with vaginal GBS have a higher likelihood of receiving multiple doses of antibiotics during labor, while their newborns are given antibiotics to treat suspected sepsis [21,22]. Although the reduced prevalence of early onset of GBS infection has been attributed to the extended use of IAP, some researchers have identified methodological bias in published studies [23]. Moreover, the potential adverse impacts of IAP on the initial colonization of gut microbiota and the interest in reducing antibiotic use globally have raised attention to alternative approaches.

2. Synthesis of evidence on infant antibiotic use and future allergic disease

Characterized by disorders such as asthma, rhinitis, conjunctivitis, urticarial and atopic dermatitis (allergic eczema), and also drug, insect, food allergy and anaphylaxis, the prevalence of allergic disease is increasing worldwide, especially, for asthma and atopic dermatitis in children and young adults [24,25]. Asthma, the most common chronic respiratory disease, and atopic dermatitis, the most common inflammatory skin disease in young children, pose significant clinical and economic burden globally [24,26]. As shown in a recent review by Sicherer et al. [27], there is a general consensus that the incidence of food allergy is also increasing. As such, there has been a long-standing interest and need for evidence on the association between infant antibiotic exposure and future allergic disease.

The first published meta-analysis by Marra et al. in 2006 [6] on the association between infant antibiotic treatment and subsequent childhood asthma found an overall pooled odds ratio (OR) of 2.05 (95% confidence interval [CI]: 1.41–2.99) in eight studies published from 1996 to 2005 (Table 1). Further meta-analysis of subgroups showed the association to be substantially stronger in cross-sectional studies (pooled OR = 2.82; 95% CI: 2.01–3.85), whereas that of prospective studies lost statistical significance (pooled OR = 1.12; 95% CI: 0.88–1.42). To reduce reverse causation bias, Murk et al.'s [7] systematic review of infant antibiotic use in 2011 was limited to studies that separated the antibiotic exposure and disease outcome by a minimum of 2 years. Their meta-analysis of 20 studies published from 1999 to 2010 produced an overall increased risk of 52% (pooled OR = 1.52; 95% CI: 1.30–1.77) for child asthma but with high heterogeneity. Similar to Marra et al. [6], cross-sectional studies produced a higher risk of asthma (pooled OR = 2.04; 95% CI: 1.83–2.27), while prospective studies minimally increased the risk (pooled OR = 1.07; 95% CI: 0.89–1.28). In a systematic review also published in 2011 [8], Penders et al. excluded cross-sectional and

retrospective case-cohort observational studies altogether. Their meta-analysis of 18 longitudinal studies published before November 2010 yielded a pooled OR of 1.27 (95% CI: 1.12–1.43) for asthma or wheeze risk. Recognizing the uncertainty of an asthma diagnosis at preschool age, Penders et al. conducted a subgroup analysis of children with asthma at a later age (5–6 years) as indicated by medication treatment; no associations were found in this subgroup. With respect to atopic dermatitis, Tsakok et al.'s [9] systematic review in 2013 showed an increased risk of 41% (pooled OR = 1.41; 95% CI: 1.30–1.53) for this atopic condition from the meta-analysis of 17 studies from 1998 to 2010. After stratification by study type (cross-sectional and prospective) and study quality, the increased risk of atopic dermatitis from antibiotics remained statistically significant and of a similar magnitude for both cross-sectional (pooled OR = 1.43; 95% CI: 1.36–1.51) and prospective studies (pooled OR = 1.40; 95% CI: 1.19–1.64).

Following publication of the aforementioned systematic reviews of childhood asthma, evidence on other allergic disease phenotypes and severity has been contributed by meta-analyses, several large cohort studies derived from regional health-care databases (to be discussed later), or from the combined analysis of individual-level data from separate cohorts (see Table 2). Combining two birth cohorts from the UK and the Netherlands initiated in the late 1990s/early 2000s, Ahmadizar et al. [28] found the risk of asthma development was twice more likely among infants treated with antibiotics (pooled OR = 2.18; 95% CI: 1.04–4.60) but no evidence for greater asthma exacerbation among children with asthma who had received antibiotics during infancy. In two studies published in 2013 and 2017 from a 2003 Swedish birth cohort, findings differed by asthma phenotype [29,30]. Antibiotics given during the first week of life were an independent risk factor for atopic asthma (asthma plus reported allergic sensitization or condition) but not non-atopic asthma in 8-year-old children (OR = 3.00; 95% CI: 1.50–6.00 versus OR = 1.30; 95% CI: 0.40–4.50) [29] and in 12-year-old children (OR = 2.22; 95% CI: 1.20–4.20 versus OR = 1.40; 95% CI: 0.50–3.40) [30]. Finally, a prospective study of 14,499 children in the UK found that infant antibiotic use was a risk factor for eczema in more economically advantaged children [31], reporting an OR (OR = 1.34; 95% CI: 1.22–1.48) which is consistent with previous estimates and an updated meta-analysis of eczema just published [32].

Recent cohort studies have also examined associations between antibiotic use during infancy and the development of food allergy and other less common atopic conditions. In 2016, a Canadian prospective birth cohort study by Batool et al. [33] found a two-fold likelihood (95% CI: 1.45–2.88) for all food allergies combined in children following postnatal antibiotic exposure. Using a nested case-control of a large prospective study, Hirsch et al. [34] assessed antibiotic treatment and subsequent milk allergy ($n = 484$ cases) and non-milk food allergy ($n = 598$ cases). They found consistent associations with infant antibiotic use, reporting elevated risks of 49% (OR = 1.49; 95% CI: 1.15–1.96) and 38% (OR = 1.38; 95% CI: 1.08–1.77) for milk and food (non-milk) allergy, respectively. The recent meta-analysis by Ahmadizar et al. [32] of three earlier studies of food allergy yielded a similar OR of 1.42,

Table 1. Systematic reviews and meta-analysis of studies on the association between early life antibiotic exposure and allergic disease.

Author (year)	No. of studies included	Study design included	Timing of Antibiotic exposure (type)	Outcomes (age)	Subgroup analysis	Results
Marra et al. (2006) [6]	8	Observational studies: retrospective and prospective	First year (not specified)	Doctor diagnosed asthma (1–18 years)	Study design (retrospective, prospective), population type (high risk, general population)	Pooled odds ratio (OR) for eight studies = 2.05 (95% CI: 1.41–2.99). Retrospective studies (n = 5) pooled OR = 2.82 (95% CI: 2.07–3.85); prospective studies (n = 3) pooled OR = 1.12 (95% CI: 0.88–1.42). High risk (n = 3) pooled OR = 1.38 (95% CI: 0.63–3.03); general population (n = 5) pooled OR = 2.64 (95% CI: 1.56–4.45) Dose-response analysis (n = 5) pooled OR = 1.16 (95% CI: 1.05–1.28)
Penders et al. (2011) [8]	21	Longitudinal observational studies: cohort and nested case-control within cohort	First year (not specified)	Wheeze and/or asthma (1–10 years)	Confounding by indication (Cbl), reverse causation (RC), outcome definition	Pooled OR for 18 studies = 1.27 (95% CI: 1.12–1.43) for wheeze/asthma. Pooled OR for studies (n = 9) with low chance of RC or Cbl = 1.12 (95% CI: 0.98–1.26). Pooled OR for studies (n = 3) with wheeze/asthma beyond 5–6 years = 1.08 (95% CI: 0.93–1.23)
Murk et al. (2011) [7]	20	Observational studies: retrospective, database, prospective	First year (broad spectrum: augmentin, cephalosporins, macrolides. Narrow spectrum: amoxicillin, cloxacillin, penicillins, sulphonamides)	Asthma (3–18 years)	Study design (retrospective, database, prospective), age of asthma onset (0–1 vs. ≥2 years), asthma diagnosis (doctor diagnosed vs. parental report), adjustment (respiratory infections, number of medical consultations, family history of asthma)	Pooled OR for 20 studies = 1.52 (95% CI: 1.30–1.77). Retrospective studies (n = 8) pooled OR = 2.04 (95% CI: 1.83–2.27); database (n = 6) pooled OR = 1.35 (95% CI: 1.12–1.62); prospective studies (n = 6) pooled OR = 1.07 (95% CI: 0.89–1.28). Dose-response analysis (n = 10) pooled OR = 1.49 (95% CI: 1.24–1.80)
Tsakok et al. (2013) [9]	17	Observational studies: birth cohort, case-control, cross-sectional	First year (broad spectrum. Narrow spectrum: penicillin)	Atopic dermatitis (0–25 years)	Study type (cross-sectional, cohort), study quality (scores <7 or >8)	Pooled OR for 17 studies = 1.41 (95% CI: 1.30–1.53). Longitudinal studies (n = 10) pooled OR = 1.40 (95% CI: 1.19–1.64); cross-sectional studies (n = 7) pooled OR = 1.43 (95% CI: 1.36–1.51). Study quality <7 pooled OR = 1.40 (95% CI: 1.27–1.56); >8 pooled OR = 1.42 (95% CI: 1.23–1.65). Dose-response analysis (n = 4) pooled OR = 1.07 (95% CI: 1.02–1.11)
Ahmadizar et al. (2018) [32]	34	Observational studies: cohort, case-control, cross-sectional	First 2 years (not specified)	Allergies: allergic rhinitis, food allergy, atopic dermatitis, positive SPT, elevated plasma IgE level	Exposure and outcome reporting: clinical and objective assessment	Allergic rhinitis (n = 22) pooled OR = 1.23 (95% CI: 1.13–1.34); atopic dermatitis (n = 22) pooled OR = 1.26 (95% CI: 1.15–1.37); food allergy (n = 3) pooled OR = 1.42 (95% CI: 1.08–1.87); positive SPT (n = 3) pooled OR = 1.01 (95% CI: 0.92–1.11); elevated plasma IgE level (n = 8) pooled OR = 0.95 (95% CI: 0.77–1.16)

Table 2. Studies examining the association between early life antibiotic exposure and allergic disease.

Author (year)	Study design (country)	Study population	Age of antibiotic exposure (type)	Outcomes (age)	Method of exposure assessment (outcome assessment)	Variables identified	Results for association between antibiotic exposure and allergic disease (95% CI)	Results according to class of antibiotic (95% CI)
Jedrychowski et al. (2011) [11]	Prospective birth cohort (United States)	310 children	First 2 years (broad spectrum: cephalosporins, macrolides. Narrow spectrum: penicillins)	Asthma, wheeze (5 years)	Interview (parental report of doctor diagnosis)	Respiratory infections, gender, maternal atopy, parity, prenatal and postnatal environmental tobacco smoke	Asthma aOR = 1.65 (0.93–2.93). Wheeze aIRR = 2.17 (1.57–2.99)	Asthma and macrolide aOR = 2.14 (1.16–3.95), cephalosporin aOR = 1.98 (1.14–3.37), penicillin aOR = 1.05 (0.60–1.84). Wheeze and macrolide aIRR = 1.91 (1.12–3.27), cephalosporin aIRR = 1.08 (0.77–1.52), penicillin aIRR = 1.33 (0.88–2.03)
Goksor et al. (2013) [29]	Prospective birth cohort (Sweden)	5654 children born in 2003	First week (not specified)	Current, atopic and non-atopic asthma (8 years)	Questionnaire (questionnaire reported doctor diagnosis)	Maternal smoking during pregnancy, mother's socioeconomic status, any breastfeeding for ≥ 4 months, preterm birth, birth mode, weight	Current asthma aOR = 2.30 (1.20–4.20), atopic asthma aOR = 3.00 (1.50–6.00), non-atopic asthma aOR = 1.30 (0.40–4.50)	N/A
Wang et al. (2013) [37]	Prospective birth cohort (China: Taiwan)	263,620 children born in 1998 from NHIRD and 9910 children born in 2003 from LHID	First year (broad spectrum: tetracyclines, amphenicols, quinolones, sulfonamides and trimethoprim, beta-lactam antibiotics, lincosamides and streptogramins. Narrow spectrum: aminoglycosides, penicillin)	Atopic dermatitis, asthma, allergic rhinitis (2–6 years)	Database prescription codes (database diagnostic codes)	Gender, enrollee category at birth, geographical birth area, health-care utilization	For 1998 cohort: antibiotics and atopic dermatitis aHR = 1.61 (1.53–1.70), asthma aHR = 1.38 (1.32–1.46), allergic rhinitis aHR = 1.41 (1.35–1.47). For 2003 cohort: atopic dermatitis aHR = 1.03 (0.73–1.44), asthma aHR = 0.96 (0.72–1.29), allergic rhinitis aHR = 1.25 (0.99–1.59). Dose-response relationship observed.	Not reported
Hoskin-Parr et al. (2013) [36]	Prospective birth cohort (United Kingdom)	5780 children from ALSPAC	First 2 years (not specified)	Asthma, allergic eczema, allergic rhinitis (7.5 years)	Questionnaire (parental report, doctor diagnosis, skin prick test)	Sex, ethnicity, age, birth factors, mother's socioeconomic and lifestyle factors	Asthma aOR = 1.75 (1.40–2.17). Dose-response observed: 1 course aOR = 1.11 (0.84–1.48), 2 aOR = 1.50 (1.14–1.98), 3 aOR = 1.79 (1.34–2.40), ≥ 4 aOR = 2.82 (2.19–3.63). Allergic rhinitis aOR = 1.28 (1.03–1.60). Atopic dermatitis aOR = 1.20 (1.02–1.41). No association observed with atopy by skin prick test	N/A
Metsäälä et al. (2014) [41]	Nested case-control (Finland)	6690 pairs of children born between 1996 and 2004	First year (broad spectrum: cephalosporins, macrolides, sulphonamides and trimethoprim. Narrow spectrum: amoxicillin)	Asthma (3–9 years)	Database prescription codes (database diagnostic and prescription codes)	Maternal asthma, previous deliveries, gestational age	Asthma aOR = 1.60 (1.48–1.73). Dose-response observed. Antibiotics against gram +ve bacteria, aOR = 1.56 (1.44–1.70), UTI antibiotic aOR = 1.61 (0.90–2.87)	Asthma and cephalosporin aOR = 1.79 (1.59–2.01), macrolide aOR = 1.61 (1.46–1.78), sulphonamide and trimethoprim aOR = 1.65 (1.34–2.02), amoxicillin aOR = 1.46 (1.35–1.58), phenoxymethyl penicillin aOR = 1.27 (1.02–1.57)
Örtqvist et al. (2014) [51]	Prospective birth cohort (Sweden)	Initial cohort: 493,785 children born between 2006 and 2010. Sibling cohort: 180,894 children	First 2 years (respiratory tract: amoxicillin, penicillin, cephalosporin, macrolides. Urinary tract or skin: pivmecillinam, trimethoprim, sulphonamide, quinolones, nitrofurantoin, penicillins)	Asthma (after 2 years)	Database prescription codes (database diagnostic and prescription codes)	Infant factors: sex, birth weight, mode of delivery, respiratory diagnosis as newborn, prenatal antibiotic. Maternal factors: Dose-response observed. Sibling cohort: asthma age at delivery, family situation, birth country, education, age	Initial cohort: asthma aHR = 3.71 (3.41–4.03). Asthma and airway antibiotic aHR = 4.12 (3.78–4.50). UTI/skin antibiotic aHR = 1.54 (1.24–1.92). Dose-response observed. Sibling cohort: asthma aHR = 2.11 (1.61–2.76). Airway antibiotic aHR = 2.36 (1.78–3.13). UTI/skin antibiotic aHR = 0.85 (0.47–1.55)	Not reported

(Continued)

Table 2. (Continued).

Author (year)	Study design (country)	Study population	Age of antibiotic exposure (type)	Outcomes (age)	Method of exposure assessment (outcome assessment)	Variables identified	Results for association between antibiotic exposure and allergic disease (95% CI)	Results according to class of antibiotic (95% CI)
Wohl et al. (2015) [43]	Retrospective birth cohort (Europe: England, Germany, Austria)	507 mother-child pairs from 1996 to 2008	Perinatal and first 2 years (broad spectrum: macrolides, cephalosporins. Narrow spectrum: penicillins, aminoglycosides)	Atopic dermatitis ($\leq 2-10$ years)	Medical records, questionnaire (medical report)	Family history, pets, smoking, siblings	IAP and atopic dermatitis RR = 1.03 (0.75–1.41). IAP >24 h RR = 1.99 (1.13–3.49). No significant association with infant exposure, RR = 1.26 (0.90–1.77)	Not reported
Sun et al. (2015) [40]	Multicenter prospective birth cohort (Europe: England, Germany, Austria)	696 children enrolled in SPACE	First year (broad spectrum: cephalosporins, macrolides. Narrow spectrum: ampicillin, penicillin, trimethoprim)	Wheeze (3 years)	Questionnaire (questionnaire, parental report)	Gender, birth order, socioeconomic status, breastfeeding >6 months, tobacco smoke exposure, family history of asthma, respiratory infection	Not reported	Macrolide and wheeze aRR = 1.09 (1.05–1.13). No association observed with wheeze and other antibiotics
Pitter et al. (2016) [39]	Retrospective birth cohort (Italy: Friuli-Venezia Giulia)	143,163 children born between 1995 and 2011	First year (broad spectrum: cephalosporins, macrolides. Narrow spectrum: penicillins. Others: quinolones, sulphonamides, glycopeptides, lincosamides)	New-onset and current asthma (above 1–18 years)	Database prescription codes (database prescription records for asthma)	Child factors: sex, birth year, gestational age, birth weight, hospitalization for respiratory infections. Maternal factors: age at delivery, education	New-onset asthma aIRR = 1.50 (1.46–1.53). Dose-response observed. Current asthma at ≥ 6 years aIRR = 1.35 (1.30–1.41), ≥ 13 years aIRR = 1.19 (1.08–1.33)	New-onset asthma and cephalosporin aIRR = 1.36 (1.32–1.40), macrolide aIRR = 1.57 (1.52–1.63), penicillin aIRR = 1.43 (1.40–1.46), other antibiotics aIRR = 1.22 (1.11–1.35). Significant positive association with all antibiotics at ≥ 6 years. Significant positive association with only penicillin and macrolide at ≥ 13 years.
Wu et al. (2016) [44]	Prospective birth cohort (United States)	136,098 mother-child dyads born between 1995 and 2003 enrolled in PRIMA	Perinatal and first year (not specified)	Asthma (4–5–6 years)	Database codes, medical records, prescription claims (database diagnostic and prescription codes)	Maternal factors: smoking during pregnancy, asthma status, age at delivery, education level. Child factors: gestational age, birth weight, race, gender, severe disease	Maternal GBS (IAP) and asthma aOR = 1.03 (0.96–1.10). Infant antibiotic and asthma = OR 2.06 (1.98–2.15). For every additional course of infant antibiotic, aOR = 1.16 (1.15–1.17) for asthma risk	N/A
Batool et al. (2016) [33]	Prospective birth cohort (Canada: Hamilton and Burlington)	901 babies enrolled in FAMILY	First year (not specified)	Atopy, allergic rhinitis, wheezing, asthma, atopic dermatitis, food allergy, cow's milk allergy (1 year)	Questionnaire (doctor diagnosed parental report, skin prick test)	Prenatal and early childhood environmental exposures, maternal nutrition during pregnancy, breastfeeding, infant use of medication	Atopy OR = 2.04 (1.45–2.88)	N/A
Hirsch et al. (2016) [34]	Sex- and age-matched case-control (Pennsylvania: Geisinger Clinic)	Longitudinal data from 2001 to 2012 on 30,060 children	From birth to 30 or 60 days before diagnosis depending on allergy (broad spectrum: cephalosporins, macrolides. Narrow spectrum: penicillin)	Milk allergy, non-milk food allergy, other allergies (2 months to 7 years)	Database prescription orders (database diagnostic codes)	Race/ethnicity, history of medical assistance	At 7 years: 1–2 antibiotic orders and milk allergy aOR = 1.49 (1.15–1.96), food allergy aOR = 1.38 (1.08–1.77), other allergies aOR = 1.69 (1.50–1.92), ≥ 3 and milk allergy aOR = 1.78 (1.28–2.48), food allergy aOR = 1.65 (1.27–2.14), other allergies aOR = 3.07 (2.72–3.46).	At 7 years: cephalosporin and milk allergy aOR = 1.40 (1.02–1.93), food allergy aOR = 1.57 (1.29–1.93), other allergy aOR = 1.70 (1.57–1.83). Macrolide and milk allergy aOR = 1.68 (1.19–2.37), food allergy aOR = 1.58 (1.28–1.96), other allergies aOR = 1.98 (1.83–2.15). Penicillin and milk allergy aOR = 1.58 (1.24–2.02), food allergy aOR = 1.29 (1.04–1.59), other allergies aOR = 2.28 (2.06–2.53)
Taylor-Robinson et al. (2016) [31]	Longitudinal cohort (United Kingdom)	14,499 children born between September 2000 and January 2002 from the U. K. MCS	First year (not specified)	Atopic dermatitis (5 years)	Survey interview (questionnaire, parental report)	Maternal, perinatal, postnatal factors	Atopic dermatitis OR = 1.34 (1.22–1.48)	N/A

(Continued)

Table 2. (Continued).

Author (year)	Study design (country)	Study population	Age of antibiotic exposure (type)	Outcomes (age)	Method of exposure assessment (outcome assessment)	Variables identified	Results for association between antibiotic exposure and allergic disease (95% CI)	Results according to class of antibiotic (95% CI)
Ahmadizar et al. (2017) [28]	Cohort (United Kingdom; Netherlands and Scotland)	Population cohort: 7393 children (Generation R), 891 children (SEATON), Asthma cohort: 668 children (PACMAN), 806 children (BREATHE)	First 3 years (not specified)	Asthma, asthma exacerbations (4–19 years)	Questionnaire, database prescription codes (questionnaire reported doctor diagnosis)	Age, gender, family history of asthma	Population cohort: asthma pooled OR = 2.18 (1.04–4.60). Asthma cohort: asthma exacerbation pooled OR = 0.93 (0.65–1.32). No significant dose–response observed	N/A
Yamamoto-Hanada et al. (2017) [38]	Prospective birth cohort (Japan)	1550 newborns registered from March 2004 to August 2006 from T-CHILD	First 2 years (broad spectrum: cephem, macrolides, Narrow spectrum: penicillins)	Allergic diseases: asthma, wheeze, atopic dermatitis, allergic rhinitis (5 years)	Questionnaire (questionnaire, parental report)	Maternal factors: history of allergy, education level, age at pregnancy, BMI, previous live births, smoking during pregnancy, Child factors: delivery mode, gestational age, day-care, bronchitis, sex	Current asthma aOR = 1.72 (1.10–2.70), current atopic dermatitis aOR = 1.40 (1.01–1.94), current allergic rhinitis aOR = 1.65 (1.05–2.58), current wheeze aOR = 1.24 (0.86–1.78)	Cephem and current asthma aOR = 1.97 (1.23–3.16), current allergic rhinitis aOR = 1.82 (1.12–2.93), no association with other allergies. Macrolide and current atopic dermatitis aOR = 1.58 (1.07–2.33), no association with other allergies. No association with penicillin and allergy
Stromberg Celind et al. (2018) [30]	Prospective birth cohort (Sweden)	5654 children born in 2003	First week (not specified)	Current, atopic and non-atopic asthma (12 years)	Questionnaire (questionnaire reported doctor diagnosis)	Prenatal smoking exposure, parents socioeconomic status, preterm birth, weight, breastfeeding ≥4 months	Current asthma aOR = 1.90 (1.10–3.20), atopic asthma aOR = 2.20 (1.20–4.20), non-atopic asthma aOR = 1.40 (0.50–3.40)	N/A
Yoshida et al. (2018) [14]	Retrospective cohort (Japan)	Initial cohort: 83,470 children born between January 2005 and September 2014, Sibling cohort: 28,085	First year (broad spectrum: cephalosporins, macrolides, Narrow spectrum: penicillins)	Asthma (1–6 years)	Database prescription codes (database diagnostic and prescription codes)	Initial cohort: sex, Sibling cohort: sex, siblings	Initial cohort: asthma at 1–3 years aHR = 2.43 (2.20–2.69); asthma aHR at 3–6 years = 1.23 (1.11–1.36). Sibling cohort: asthma at 3–6 years aHR = 1.62 (1.27–2.07). Dose–response observed in both cohorts	Initial cohort: asthma at 1–3 years and cephalosporin aHR = 1.55 (1.41–1.70), macrolide aHR = 2.00 (1.83–2.20) penicillin aHR = 1.30 (1.26–1.53); asthma at 3–6 years and cephalosporin aHR = 1.20 (1.08–1.33), macrolide aHR = 1.17 (1.05–1.31), penicillin aHR = 0.87 (0.76–0.98). Sibling cohort: asthma and cephalosporin aHR = 1.35 (1.07–1.71), macrolide aHR = 1.48 (1.17–1.87), penicillin aHR = 1.28 (0.97–1.69)
Mitre et al. (2018) [35]	Retrospective cohort (United States)	792,130 children with a birth medical record in the Military Health System database between October 2001 and September 2013	First 6 months (broad spectrum: macrolides, cephalosporins, amoxicillin clavulanate, trimethoprim with sulfamethoxazole, Narrow spectrum: penicillin, amoxicillin)	Allergic diseases: asthma, atopic dermatitis, food allergy, allergic rhinitis, allergic conjunctivitis, urticaria, contact dermatitis, anaphylaxis, medication allergy (after 6 months)	Medical record database (database diagnostic codes)	Prematurity, cesarean delivery, sex, other drug classes	Asthma aHR = 2.09 (2.05–2.13), atopic dermatitis aHR = 1.18 (1.16–1.19), allergic rhinitis aHR = 1.75 (1.72–1.78), allergic conjunctivitis aHR = 1.42 (1.34–1.50), food allergy aHR = 1.14 (1.10–1.18), anaphylaxis aHR = 1.51 (1.38–1.66), medication allergy aHR = 1.34 (1.29–1.40)	Not reported

OR: odds ratio; aOR: adjusted odds ratio; aHR: adjusted hazards ratio; RR: relative risk; aRR: adjusted relative risk; aIRR: adjusted incident rate ratio; NHIRD: National Health Insurance Research Database; LHID: Longitudinal Health Insurance Database; ALSPAC: Avon Longitudinal Study of Parents and Children; SPACE: Study of Prevention of Asthma in Children in Europe; PRIMA: Prevention of RSV: Impact on Morbidity and Asthma; FAMILY: Family Atherosclerosis Monitoring In early life; U.K. MCS: United Kingdom Millennium Cohort Study; SEATON: Study of Eczema and Asthma To Observe the influence of Nutrition; PACMAN: Pharmacogenetics of Asthma Medication in Children: Medication with Anti-inflammatory Effects; T-CHILD: Tokyo Children's Health, Illness and Development Study.

95% CI: 1.08–1.87. Adding to this evidence, Mitre et al. [35] recently determined the risk of postnatal antibiotic use against a wide array of allergic disease in a retrospective cohort analysis of the health-care database records of almost 800,000 children in the United States. They found significant associations in their adjusted analysis for food allergy (hazard ratio [HR] = 1.14; 95% CI: 1.10–1.18), anaphylaxis (HR = 1.51; 95% CI: 1.38–1.66) and even medication allergy (HR = 1.34; 95% CI: 1.29–1.40). Associations were also found for asthma (HR = 2.09; 95% CI: 2.05–2.13) and atopic dermatitis (HR = 1.18; 95% CI: 1.16–1.19).

There is strong evidence of dose-dependence on the risk of childhood atopy following the antibiotic treatment of infants. The pooled dose-response from the meta-analysis by Tsakok et al. [20] of four studies gave a 7% increased risk for eczema (95% CI: 1.02–1.11) with each further course of antibiotic treatment. In 2013, investigators of the ALSPAC birth cohort reported dose-dependence between antibiotic treatment assessed until age 2 and subsequent asthma risk [36]. After adjusting for covariates, the OR for child asthma in decreasing magnitude of exposure was 2.82 (95% CI: 2.19–3.63), 1.79 (95% CI: 1.34–2.40), 1.50 (95% CI: 1.14–1.98), 1.11 (95% CI: 0.84–1.48) for antibiotics taken four times or more, thrice, twice, and once, respectively. Highest associations were found when antibiotics were administered both during infancy and the year afterwards until age 2. Excluding infants with early wheeze who had asthma by age 8 (to address reverse causation), a statistical association was only retained with four or more courses of antibiotic treatment (OR = 1.65; 95% CI: 1.16–2.35) [36]. Following almost 270,000 infants born in 1998 and 10,000 infants born in 2003 for 6 years, a recent publication from Wang et al. [37] also found dose-dependence with infant antibiotic use but this varied by the year of the cohort. In the 1998 cohort, more than four courses of antibiotic treatment increased the risk of asthma (adjusted HR = 1.17; 95% CI: 1.14–1.20) and atopic dermatitis (adjusted HR = 1.16; 95% CI: 1.14–1.18), while in the 2003 cohort, no statistical associations were evident with either condition. Certainly, retrospective reporting of infant antibiotic courses greatly overestimates risk in cross-sectional studies. The dose-dependent association from birth cohort studies is suggestive of causation and implies that a cumulative exposure may be more important than a single exposure in determining allergic disease outcomes.

Antibiotics affect the microbial composition of the infant gut according to their spectrum of activity. For a summary of microbial groups typically affected, refer to Table 1 in the Kozyrskyj et al.'s book chapter [38]. The impact of infant exposure to antibiotics on later atopy development appears to be linked to the spectrum of the antibiotic, with varying associations reported for broad-spectrum and narrow-spectrum antibiotics. In a small study of 310 children, Jedrychowski et al. [11] found that while use of any type of antibiotic was not associated with increased risk of asthma, sub-analysis by antibiotic type revealed an increased risk of asthma with exposure to macrolides (adjusted odds ratio [aOR] = 2.14; 95% CI: 1.16–3.95) and cephalosporin (aOR = 1.98; 95% CI: 1.14–3.37), but not penicillin. In the subgroup analysis of an Asian birth cohort with 1550 participants, cephem antibiotic was positively associated with asthma (OR = 1.97; 95% CI: 1.23–3.16) and allergic rhinitis

(OR = 1.82; 95% CI: 1.12–2.93), macrolide antibiotics were associated with atopic dermatitis (OR = 1.58; 95% CI: 1.07–2.33), while penicillins showed no impact on the risk of allergies [39]. Pitter et al. [40] investigated a longitudinal cohort of 143,000 children in Italy to find that antibiotic exposure in first year of life increased the risk of new-onset asthma by 51% (incident rate ratio [IRR] = 1.51; 95% CI: 1.48–1.54). The association in this large study differed across classes of antibiotics, with macrolides having the greatest association (IRR = 1.57; 95% CI: 1.52–1.63) compared to cephalosporins (IRR = 1.36; 95% CI: 1.32–1.40) and penicillins (IRR = 1.43; 95% CI: 1.40–1.46). In addition, a recent multi-center birth cohort study by Sun et al. [41] of high atopic-risk children found a slight risk of wheeze following macrolide antibiotic use (relative risk [RR] = 1.09; 95% CI: 1.05–1.13) but not with cephalosporin and penicillin treatment after adjusting for covariates. Using a nested-case control study, Metsala and colleagues [42] were able to detect a statistically significant association between asthma and narrow-spectrum phenoxymethylpenicillin (OR = 1.27; 95% CI: 1.02–1.57) and amoxicillin (OR = 1.46; 95% CI: 1.35–1.58). When grouped by indication, they found that the antibiotic treatment of gram-positive infections significantly elevated asthma risk, while those for gram-negative bacteria infection showed no association. Rather surprisingly, Yoshida et al. [14] found a significant protective effect for asthma at age 3 with penicillin treatment of the infant (HR = 0.87; 95% CI: 0.76–0.98).

Timing of the antibiotic exposure may also be important. As noted in the introduction, maternal IAP for GBS prevention is another source of antibiotic exposure for the newborn that could influence the risk of allergy, but this area is less well researched [43]. Wohl and colleagues [44] found that among women who received IAP, there was significant risk of atopic dermatitis (RR = 1.99; 95% CI: 1.13–3.49) in their infants only when the exposure to IAP was greater than 24 h. Additionally, Wu et al. [45] demonstrated an increased odds of childhood asthma from perinatal antibiotics, including IAP, that lost significance after adjusting for covariates (OR changed from 1.27; 95% CI: 1.19–1.35 to 1.03; 95% CI: 0.96–1.10).

To sum, the body of evidence from published meta-analyses and epidemiologic studies on antibiotic exposure during infancy is subject to the heterogeneity of allergic disease definitions, retrospective versus prospective reporting of antibiotic use, and several methodologic biases related to reverse causation and confounding by indication. The latter will be subject to a more detailed discussion in the next section, including a closer examination of the period effect of the Wang et al. study [37]. While study bias may lead to overestimated associations, general opinion is that a causal link between infant antibiotic exposure, especially to broad-spectrum antibiotics or repeated courses, and allergic disease likely exists. When compared to other early life factors, infant antibiotic treatment appears to have the greatest association with asthma development [35,45]. With increased sample size from the publication of additional studies and capacity to adjust for a greater range of confounding factors (see Table 2), new systematic reviews and meta-analyses will have greater power to conduct sub-analyses, and tackle study bias and issues of causation. Most newer studies account for major

confounding factors, such as maternal smoking or breastfeeding status (Table 2). However, the external microbial environment (i.e. rural or farm residence) or factors affecting the home microbial environment such as siblings, pets, and household cleaning products should not be overlooked. As has been done to a limited degree [46], the modifying effects of the microbial environment and other confounding factors on infant antibiotic use should also be tested.

3. Methods to reduce bias in reported associations

Remaining to be resolved are two main biases which affect reported associations between infant antibiotic treatment and the development of allergic disease: reverse causation and confounding by indication. As it implies, reverse causation bias indicates that the antibiotic may have been administered to the infant after the diagnosis of an allergic condition (e.g. atopic dermatitis/eczema). Confounding by indication refers to the presence of a third factor (e.g. respiratory symptoms) which is associated both with infant antibiotic treatment and asthma development. Noted earlier, retrospective questionnaire studies are unable to discern temporality and typically report the highest associations between infant antibiotic use and future allergic disease. The meta-analyses by Murk et al. [7] and Penders et al. [8] have reported reduced or null associations for prospective studies which have controlled for these two biases. However, it is also challenging to design prospective studies which ascertain in detail dates for the first antibiotic prescription and for the first sign of symptoms. New parents may have difficulty remembering these details, although according to the results of prospective studies in Table 2, similar associations are found between studies which rely on parent recall of infant antibiotic treatment versus those based on prescription record sources. A meta-analysis which stratifies prospective studies by method of antibiotic use measurement (parent report versus dispensed prescription records) is yet to be published. In this section, we will discuss how reverse causation and confounding by indication biases have been further addressed in studies to date.

Evaluating early antibiotic treatment of the infant, for example, soon after birth and before the onset of symptoms associated with allergic disease is an optimal strategy to prevent reverse causation bias. Indeed, associations between newborn antibiotic treatment (following admission to a neonatal unit after birth) and preschool wheeze or asthma have been reported in several studies [47,48]. In their study of intravenous penicillin/ampicillin and gentamicin regimens in newborns suspected of sepsis [49], Sobko et al. noted a borderline 1.5-fold association between intravenous antibiotic treatment and asthma in older children (median age, 12). A statistically significant OR was found for confirmed neonatal sepsis, which would have required intravenous antibiotic treatment. Analyses were adjusted for gestational age to reduce bias from the influence of preterm birth.

Addressing reverse causation bias does not take care of issues related to confounding by indication. This is because respiratory conditions, which could indicate future asthma or other allergic disease, are the major symptoms of infection in infants and indication for antibiotic treatment. For example,

infants born by scheduled cesarean are more likely to have respiratory distress [50]. Hence, a common strategy is to exclude infants with respiratory illness episodes during the first year of life or prior to the first antibiotic prescription, or adjust for this factor. In the Murk et al.'s meta-analysis [7], adjustment for respiratory infection diminished the infant antibiotic association with child asthma. Other researchers have tested associations with infant antibiotic use for non-respiratory infections. In earlier work, Marra et al. [51] and Kozyskyj et al. [46] found associations between childhood asthma and infant antibiotic treatment for non-respiratory indications, such as skin and urinary tract infections. More recently in a nationwide database study, Ortqvist et al. [52] observed associations between infant antibiotic treatment of skin and urinary tract infections, and child asthma; they were of a higher magnitude (HR of 1.5) when the antibiotic was administered within 5 months of birth. Noteworthy is that all associations between infant antibiotic treatment and childhood asthma, including those for non-respiratory tract infections, became statistically nonsignificant in the sibling component of the analysis. Authors attributed this loss of association to a heavy influence of familial factors, including genetic predisposition [53], which has been reported by others in connection with impaired antiviral immunity.

Of potential relevance to confounding by indication is acetaminophen therapy since acetaminophen is frequently administered concurrently with an antibiotic for the treatment of pain or fever in ear or respiratory infections. Wang et al. [37] found that combined treatment of the infant with antibiotics and acetaminophen yielded the greatest association with child asthma. Of particular interest in their study is that the HR for combined and singular exposure to antibiotics or acetaminophen was lower in the 2003 cohort (HR = 1.66; 95% CI: 1.44–1.92) versus a 1998 cohort (HR = 2.33; 95% CI: 2.24–2.43) in Taiwan. In contrast, HRs for the main indications for infant antibiotic treatment, ear infection, and bronchitis rose from 1998 to 2003. With concerted efforts to reduce unnecessary antibiotic treatment of viral bronchitis and ear infection over the last three decades, these discrepant findings for measures of antibiotic utilization versus indication for treatment again point to the strong influence of confounding by indication in earlier studies. On the other hand, more judicious use of antibiotics expected in recent cohorts may diminish this bias and identify a causal association for antibiotic treatment.

Preexisting infection may be another example of confounding by indication for associations with infant antibiotic treatment, as infection during infancy may lead to asthma development. The Sobko et al. [49] study of neonatal antibiotics implemented a design feature to adjust for neonatal infection, the testing of antibiotic regimens for suspected but not confirmed sepsis. As noted, this study documented a borderline association between antibiotic treatment 'to rule out sepsis' and childhood asthma, but it was of a stronger magnitude than that for confirmed sepsis following adjustment for multiple perinatal factors [49]. Another newborn condition of full-term infants, which is frequently treated with antibiotics but may not involve infection, is transient tachypnea of the newborn (TTN). Several studies have reported associations between TTN and future asthma [54,55]. However, the prevalence of TTN is much lower than that for childhood

asthma and the presence of respiratory symptoms may also be a sign of subsequent respiratory illness. Infection may commence in utero. In their study of early life exposures in a US cohort born between 1995 and 2003 [45], Wu et al. found the greatest association between child asthma and multiple courses of antibiotics (eight or more) to be in the subgroup of infants who were delivered by CS following repeated urinary tract infection in pregnancy. This association was further increased in the absence of siblings at home (aOR = 7.77; 95% CI: 6.25–9.65).

4. Can microbiome studies address confounding by indication?

It is well known that antibiotic treatment perturbs gut microbiota, especially in infants and young children [38], evidence which is reaffirmed with large-scale gene sequencing studies of the gut microbiome. Temporal assessment of gut microbial composition in relation to antibiotic treatment and before the onset of allergic disease has been proposed to disentangle bias from reverse causation and confounding by indication [56]. To effectively do this, microbiota studies require timed collection of fecal samples, detailed recording of antibiotic regimens, and ample sample size to follow infants who are antibiotic-free prior to the first antibiotic prescription or indirect exposure from the mother, and homogenous for other perinatal factors that affect gut microbiota, such as birth and breastfeeding mode. Knowing the infant age at time of antibiotic administration is critical to take into account the ecological successions during infancy, seen as a predominance of *Enterobacteriaceae* soon after birth, followed by a rise in the facultative lactic acid bacteria, then strict anaerobic bacteria over the next few months [57]. Paying attention to the temporality of events, Verhulst et al. [58] confirmed that antibiotic treatment during the first 3 weeks of life was not associated with wheezing throughout infancy. Study infants were born vaginally at term gestation and 60% were breastfed at 3 weeks of age. Only antibiotic use at 6 months was cross-sectionally correlated with wheeze at the same age, pointing to the likelihood of reverse causation in this association. Consistent with the Tanaka et al.'s [59] study (that will be discussed later) but based on culture methods, they found an association between wheeze episodes during infancy and total anaerobic concentrations in fecal samples obtained 3 weeks after birth. This association was independent of infant antibiotic use, which they had established to be a marker of wheeze.

Evaluating antibiotic treatment soon after birth permits one to detect change in microbial composition which can be attributed to the antibiotic before the onset of signs or symptoms. Antibiotics are commonly administered medications soon after birth, especially in neonatal intensive care units [60]. Preterm babies receive broad-spectrum antibiotics, typically used for empirical therapy [61]. However, extensive exposure to antibiotics is not restricted to preterm babies; in some populations more than half of infants have been treated with antibiotics during the first months of life [62]. Common perturbations secondary to the oral and intravenous antibiotic treatment of the neonate immediately after birth are reduced fecal abundance of genus *Bifidobacterium* and increased *Enterobacteriaceae* [63–65]. Full recovery of this gut dysbiosis after the cessation of antibiotic

treatment does not occur [63,65]. In the study of Japanese newborns treated with oral cephalixin immediately after birth, Tanaka et al. [63] observed lower microbial diversity which persisted in the infant gut at 2 months, accompanied by reduced abundance of bifidobacteria, overgrowth of enterococci, and subsequent enrichment with the *Enterobacteriaceae*. Disease outcomes were not reported in this study. More recently [59], the gut microbiota of Japanese infants who developed asthma, food allergy, or other allergic condition by age 3 years were found to be enriched with *Enterobacteriaceae* at age 1 compared to those of infants who did not develop allergic disease. Of note, the greater likelihood of food allergy following increased fecal abundance of *Enterobacteriaceae* was found to be independent of antibiotic treatment. In this later study, the vast majority of antibiotic use in infants was recorded to occur between 6 and 12 months after birth and not before then.

What is the evidence that adjustment for early antibiotic exposure 'explains' reported associations between gut dysbiosis and future allergic disease? According to the CHILd birth cohort study [66], observed gut dysbiosis in 3-month-old infants, documented in fecal samples as reduced microbial species richness and a higher ratio in the abundance of the *Enterobacteriaceae* versus *Bacteroidaceae* (E/B ratio), predicted food sensitization at age 1 (a marker of future asthma). The E/B ratio findings have since been replicated by two studies, of which Chen et al. also reported genus level results, namely enrichment by *Suterella* (of *Enterobacteriaceae*) and depletion of *Bacteroides* (of *Bacteroidaceae*) [67,68]. In the CHILd study, the E/B ratio association was independent of infant antibiotic exposure (direct or indirect from the mother at birth) by 3 months of age and also of birth mode. Like the aforementioned study of Japanese infants, these findings hint at the possibility that allergic disease risk following early perturbation of the gut microbiome is not related to antibiotic use. Others have reported that infant gut dysbiosis also precedes allergic disease in cohort studies that exclude antibiotic exposure within 1 month of birth [69]. As such, the current microbiome literature supports explanations of reverse causation or confounding by indication. On the other hand in the CHILd study, reported associations with the E/B ratio did not survive adjustment for breastfeeding [66]. In view of the stronger associations between multiple courses of antibiotics during infancy and childhood asthma [7], oral antibiotic use in later infancy after cessation of breastfeeding also needs to be examined more closely. Korpela et al. [70] documented greater associations between altered abundance of specific microbial taxa and infant body mass index (BMI) with an increasing number of antibiotic courses. We are unaware of an equivalent paper on risk for asthma/atopy development.

5. New insights – antibiotic-induced changes to the resistome

Early life antibiotic exposure affects not only the composition of gut microbiota in infants but also the carriage of resistance genes by gut microbes. Called the human gut resistome, this entity is defined as the collection of all resistance genes on microbial chromosomes in the gut microbiome [71,72]. Antimicrobial resistance is a global public health concern, so it is surprising that few

studies have been conducted on the resistome of gut microbiota [73]. Evidence of antibiotic resistance is also a specific marker of antibiotic treatment, which is valuable when antibiotic use status of the infant is unknown. Indirect exposure via the mother during birth or afterwards is often overlooked and is worth reviewing. Human studies have shown that maternal IAP significantly affects gut microbial composition of the newborn [74], potentially due to reduced transmission of lactobacilli during vaginal birth [75], specifically to newborn oral microbiota [76]. At 3 months of age, Azad et al. [77] reported that maternal IAP significantly reduced the abundance of genera *Bacteroides* and *Parabacteroides*, while it increased *Enterococcus* and *Clostridium*, microbes which commonly emerge following prophylaxis with beta-lactam antibiotics. The impact of birth mode on gut microbiota has been identified as more prominent than repeated infant antibiotic treatment [78], but given the extensive use of maternal IAP, especially in CS delivery, birth mode associations are always confounded with antibiotic treatment. Hence, gut dysbiosis in CS-delivered babies should always be viewed as a partial consequence of maternal IAP [57,65,79,80]. Finally, there is emerging evidence that maternal antibiotic treatment significantly alters breast milk microbiota [81], which can result in infant consumption of dysbiotic milk microbiota, as well as trace amounts of antibiotics in mother's milk. In fact, Azad et al. [77] documented maternal antibiotic treatment to be more common after emergency cesarean when compared to vaginal birth or scheduled cesarean, and observed a greater extent of gut dysbiosis in infants delivered by emergency cesarean.

As noted, we know little about the impact of early life antibiotics in shaping the resistome of infant gut microbiota, promoting the overgrowth of pathogenic microbes and compromising clinical management of infection, and increasing susceptibility to chronic disease [73]. Several studies have certainly demonstrated the existence of resistance genes in early life microbiota and after antibiotic treatment [82–85]. In a longitudinal follow-up by Yassour et al. [85], antibiotic resistance genes peaked in abundance after an infant oral antibiotic treatment course (with mostly penicillins but also macrolides and sulfonamides for ear infections) followed by a sharp decline, whereas some genes persisted longer. Following maternal IAP, a recent report by Nogacka et al. [82] detected the enrichment of genes conferring resistance to β -lactam antibiotics together with increases to Proteobacteria and Firmicutes. In addition, an association between maternal IAP and expression of β -lactam resistant genes in the oral microbiome of both mothers and neonates has been reported by Gomez-Arango et al. [86]. Recently, a clinical study noted fecal carriage of enterobacteria resistant to ampicillin and trimethoprim-sulfamethoxazole to be significantly higher in children with oral antibiotic exposure compared to those treated with intravenous antibiotics, indicating that the route of administration of antibiotics may also play a significant role in emergence of resistant organisms [87]. Of note, Gosalbes and coworkers reported that the meconium of more than half of the newborns harbored genes involved in resistance to β -lactam antibiotics and tetracycline [84]. The authors suggested that the vertical transmission of antibiotic resistance genes from mother to offspring could occur even prior to birth. They also proposed that resistant genes from

the mother may reach the infant gut through colostrum and other sources, such as the mother's body or the environment. Finally and importantly, others have observed the infant gut resistome to be unique from that of their mothers; it contains broad-spectrum β -lactamases which may originate from infant treatment with antibiotics or the hospital or home microbial environment [88,89].

6. New insights – antibiotic-induced changes to the fungal microbiome

The gut microbiome is composed of bacteria, viruses, phages, fungi, protozoans, and helminths; yet most studies to date have only included bacteria [90,91]. There is very limited knowledge on the composition of nonbacterial communities, but fungi are increasingly being recognized as common members of the microbiome, albeit in smaller proportion than bacteria [92]. A recent survey of the fungal microbiome (mycobiome) of 377 healthy adults enrolled in the Human Microbiome Project [93] revealed that the gut mycobiome consists mainly of yeasts (8 out of 15 most abundant genera), including *Saccharomyces*, *Malassezia*, and *Candida* species [94]. Studies in infants have shown that the early life mycobiome consists of few (generally <16 per sample) unique Operational Taxonomic Units (OTUs), including *Candida parapsilosis*, *C. tropicalis*, *C. albicans*, *Saccharomyces cerevisiae*, *C. orthopsilosis*, *Cladosporium* sp., and *Cryptococcus pseudolongus* [95]. As with bacterial microbiomes, there is a very high degree of interindividual variability [95,96]. Often, individual mycobiomes are dominated by a single fungal taxon [95,96]. Notably, a recent study in US infants found that the diversity of fungal species is particularly high during the first 4 months of life, and that it changes in reciprocal association with bacterial diversity during the first months of life [97], suggesting that the ecological patterns of microbiome diversity involve yet-to-be-defined inter-kingdom interactions [98].

There is robust evidence implicating the antibiotic treatment as an early life exposure that alters the fungal microbiome. Fungi are naturally resistant to antibiotics, and can take advantage of the disruption of bacterial communities and the subsequent decline in colonization resistance mechanisms. Fungal overgrowth is a common side effect of antibiotic treatment in neonates and infants that can manifest clinically as oral thrush and fungal diaper dermatitis [99,100]. An important study comparing the efficacy of amoxicillin-clavulanate versus placebo in the treatment of otitis media found that diaper dermatitis was the principal side effect of antibiotic treatment, occurring in 47% of antibiotic-treated infants [100]. The high occurrence of these side effects suggests that fungal overgrowth likely occurs sub-clinically as well. Evidently, antibiotic-induced bacterial perturbations in the gut can enable fungi to establish a niche, yet this has not been studied with sufficient detail using next-generation sequencing methods.

While the ecological effects of antibiotic treatment remain poorly characterized, certain fungi are known to exacerbate antibiotic-induced bacterial dysbiosis. For example, introduction of *C. albicans* to mice previously treated with antibiotics resulted in non-transient and significant shifts in the bacterial microbiome compared to changes induced by antibiotic use alone [101,102]. This resulted in changes in intestinal

homeostasis markers, such as a decrease in antimicrobial peptides and tight junction proteins, suggesting that post-antibiotic fungal blooms elicit unfavorable consequences to host immunity [101]. On the other hand, not all gut fungi have the invasive and deleterious effects described for *C. albicans*. The probiotic yeast *Saccharomyces cerevisiae* var. *boulardii* ('*S. boulardii*') is frequently used as an effective strategy to prevent antibiotic-induced diarrhea in infants, traveler's diarrhea, and diarrhea associated inflammatory bowel disease [103,104]. It has been proposed that this yeast interacts synergistically with beneficial bacteria, such as *Lactobacillus rhamnosus* [105] to inhibit the growth and expression of virulence factors of *C. albicans* [106,107]. Thus, just like the bacterial microbiome, host-fungal interactions are species- and context-dependent. Given the limited knowledge on the gut mycobiome, it is very likely that additional fungal-fungal and fungal-bacterial agonistic or antagonistic interactions readily occur. Studies on the effect of antibiotic use on the microbiome represented an ideal yet underused setting to study these ecological interactions.

Concomitantly, it is important to determine the role that antibiotic-induced fungal dysbiosis may have in mechanisms related to health and disease. Recent findings from longitudinal infant cohorts have found bacterial and fungal dysbiosis preceding [97,108,109] and/or mechanistically implicated [97,109,110] with human allergic diseases. They suggest that microbes are needed to stimulate the immune system and when they altered by antibiotic treatment, lowered abundance of regulatory immune cells and/or specific short-chain fatty acid metabolites may predispose to the development of allergy. Given the putative associations between antibiotic use and subsequent disease risk for allergic disease, it is critical to further explore the nature of these signals beyond bacteria, as well as their mechanistic interactions with the infant immune system and metabolism.

7. Conclusion

Through meta-analytic synthesis of the literature, accumulating evidence continues to support earlier findings of association between infant antibiotic treatment, especially with multiple courses and/or broad-spectrum antibiotics, and the development of allergic disease. Many studies are able to show independence of this association from reverse causation and other early life factors. More recently published studies on large infant populations report associations with the antibiotic treatment of conditions, i.e. urinary tract infection, which do not precede allergic disease. However, reducing bias from confounding by indication for the antibiotic prescription remains a challenge even in these types of studies, with emerging evidence that susceptibility to infection is a pathway to atopy. In the meantime, increasing evidence that early changes in the gut microbiome, mycobiome, or even resistome are associated with the development of allergic disease may provide a closer link to antibiotic treatment or refute it as a cause of disease development. Greater study of the resistome will also provide further evidence on the impact of widespread use of antibiotics in infant populations on the development of antibiotic resistance. In the end, this scientific evidence will

promote establishment of antibiotic stewardship programs targeted at infants and young children in hospital and community settings alike, to reduce exposure during this vulnerable time of human development [111].

8. Expert commentary

Meta-analytic evidence clearly shows that reverse causation bias, most evident in cross-sectional studies, overestimates the association between infant antibiotic treatment and future allergic disease. Finding antibiotic dose and spectrum associations with allergic disease adds biological plausibility to support a causal role for infant antibiotic treatment. However, these types of associations do not eliminate confounding by indication as a source of bias, even in cohort studies. Testing associations specific to the indication for the antibiotic, namely for non-respiratory conditions, offers a solution for this analytical challenge. The Ortqvist et al. [52] nation-wide Swedish study of ~500,000 infants is important in this regard, reporting elevated risks of 1.5 following the antibiotic treatment of skin and urinary tract infection. Yet skin conditions are potentially markers for diagnosed or undiagnosed atopic dermatitis.

With advances to gene sequencing methods to enable high throughput profiling of the gut microbiome, we now have another tool to address confounding by indication through the large-scale detection of changes to the infant gut microbiome, secondary to antibiotic treatment and before the development of allergic disease. In a limited number of studies, gut dysbiosis (e.g. changes to gut diversity and taxon abundance) prior to allergic disease are reported to occur in studies which exclude prior antibiotic exposure in 1-month infants or adjust for its presence up to 3 months after birth [58,66,69]. One explanation for the independent associations seen with both infant antibiotic treatment and gut dysbiosis in multivariable models is that the former is a marker of wheeze (and confounding by indication), while the latter is the true causal association with atopy. Of note is that these studies did not take into account antibiotic treatment in later infancy, which according to Hoskin-Parr et al. [36] especially poses a risk if there is prior antibiotic treatment. Hence, although the action of antibiotics on specific microbes is well documented, it remains unclear at this time whether changes to gut microbiota which precede disease onset are subsequent to the influence of antibiotics.

Do null associations with allergic disease truly indicate no causal role for antibiotic use during infancy? Null associations have been reported in urban (but not rural) infant populations [10,46], in the presence but not absence of maternal history of asthma [12,46] and in sibling studies that account for the genetic and other shared environments [52]. Is it possible that additional exposure of the gut microbiomes of infants already transformed by genetics [112] or a microbial environment does not confer additional risk for allergic disease? Finally, what is the role of the infant resistome, mycobiome, or virome in future susceptibility to allergic disease? These are future questions to be answered.

9. Five-year view

Five years from now we will have new knowledge on the human resistome, mycobiome, and virome. This will help us answer whether antibiotic treatment in the infant has a causal

role in susceptibility to allergic disease. With additional studies on the interplay between genetic predisposition, and the microbiome, resistome, mycobiome, and virome, we will have better insight into whether the gut microbiome of infants is shaped by genetics and the extent to which changes to the microbial environment with antibiotic use confer additional risk for allergic disease.

Key issues

- Global consumption of antibiotics has risen substantially over time, and in developed countries the increased prescribing of antibiotics, namely of broad-spectrum antibiotics, in early life has coincided with an epidemic of childhood asthma and allergic diseases.
- Through meta-analytic synthesis of the literature, there is evidence of a positive association between antibiotic exposure in infancy and later development of allergic disease; however, results have been hampered by issues of reverse causality and confounding by indication.
- New evidence from large individual or combined cohorts and regional database longitudinal studies continues to support this association, which is strengthened by the biological plausibility of stronger associations seen with multiple course and broad-spectrum antibiotic treatment.
- A major source of bias, confounding by indication, has been addressed in studies of the antibiotic treatment of conditions, i.e. urinary tract infection, which do not precede allergic disease. Large-scale gene sequencing of the infant gut microbiome has provided a further tool to reduce this bias.
- In a limited number of studies to date, microbial dysbiosis of the infant gut has been found to predict allergic disease independent of antibiotic exposure up to 3 months after birth. These studies have not accounted for antibiotic treatment in later infancy, a strong risk factor for allergic disease.
- Continued study of the infant gut microbiome, mycobiome, or even resistome will provide a closer link to antibiotic treatment or refute it as a cause of allergic disease development.

Funding

This paper is not funded.

Declaration of interest

AL. Kozyrskyj declares a SyMBIOTA Canadian Institutes of Health Research grant number 227312. MC Arrieta declares research funding from NSERC, CFI, CIHR, Alberta Health Services and The University of Calgary. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

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