Expert Opinion on Drug Safety

ISSN: 1474-0338 (Print) 1744-764X (Online) Journal homepage: https://www.tandfonline.com/loi/ieds20

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To cite this article: Sarah Milliken, Ruridh M. Allen & Ronald F. Lamont (2019): The role of antimicrobial treatment during pregnancy on the neonatal gut microbiome and the development of atopy, asthma, allergy and obesity in childhood, Expert Opinion on Drug Safety, DOI: 10.1080/14740338.2019.1579795

To link to this article: https://doi.org/10.1080/14740338.2019.1579795

Accepted author version posted online: 11 Feb 2019.

Publisher: Taylor & Francis

Journal: *Expert Opinion on Drug Safety*

DOI: 10.1080/14740338.2019.1579795

Review

The role of antimicrobial treatment during pregnancy on the neonatal gut microbiome and the development of atopy, asthma, allergy and obesity in childhood

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Abstract

Introduction*:* The use of antibiotics prenatally, during pregnancy, or neonatally may have adverse effects on the neonatal gut microbiome, and adversely affect the development of the infant immune system, leading to the development of childhood atopy, asthma, allergy and obesity.
Areas covered*:* We reviewed new evidence about vaginal eubiosis and dysbiosis from molecular-based, cultivation-independent techniques, and how this affects the neonatal gut microbiome and early development of the immune system in infants. We have considered the association between maternal use of antibiotics and the potentially beneficial role of vitamin D in the development of atopy, asthma, allergy and obesity. We have presented what efforts might be made to reduce the use of antibiotics in pregnancy. Finally, we have also addressed therapeutic interventions such as vaginal "seeding", probiotics, breastfeeding and neonatal dietary supplementation.
Expert opinion*:* The weakness of the currently available research is that insufficient assessment has been paid to confounding variables. There also remains uncertainty as to whether it is relevant that the mother suffered from the same condition such as asthma, as the purported infant outcome variable, for which she may have received antibiotics. In most studies, there is also a lack of control for the number of antibiotic courses administered, the timing of use, the use of broad spectrum or narrow range antibiotics, the indication for antibiotic use, the dose dependent nature of the effect, the class of antibiotics used, or a varying degree of risk.

Keywords: Allergy, Antibacterial, Antibiotic, Antimicrobial, Asthma, Atopy, Childhood, Obesity, and Pregnancy.

Abbreviations:

ALL	Acute Lymphoblastic Leukaemia
BMI	Body Mass Index
BV	Bacterial Vaginosis
CS	Caesarean Section
GBSEOD	Group B Streptococcus Early Onset Disease
GP	General Practitioner
NIH	National Institutes of Health
OTC	Over the Counter
PPROM	Preterm Prelabour Rupture of the Membranes
PTB	Preterm Birth
UTI	Urinary Tract Infection

Article highlights

- There is increasing evidence to link the use of antibiotics in pregnancy with subsequent childhood obesity, asthma and atopic disease. This may also apply to prenatal antibiotic use.
- Vaginal birth, breastfeeding and vitamin D may have a protective effect.
- Interventions may include breastfeeding, alterations to infant diet and/or use of probiotics.
- More research is needed before "seeding" can be fully supported.
- Future research must be more robust with respect to correcting for confounding variables and should include long-term follow up.
- Greater efforts should be made to reduce the use of antibiotics in pregnancy.

1.0 Introduction

Between 2000 and 2015 the use of antibiotics worldwide has risen by 65%, and by 114% in low-income and middle-income countries (1). The extent of the use of antibiotics during pregnancy is unknown, but best-guess estimates are somewhere between 20% and 40% (2, 3), and in the USA, it is estimated that antibiotics account for 80% of medications used by pregnant women. While antibiotics may be given with good reason, they may also have unintended adverse consequences (4). Given the ubiquitous nature of antibiotic use in pregnancy, even a small adverse effect of maternal antibiotic use, may pose a substantial public health problem (5). Due to the physiological adaptations of pregnancy, urinary tract infections (UTI) and vulvovaginal candidiasis are more common, and along with upper respiratory tract infections, account for around 72% of all treated infections in pregnancy (6). Approximately 5-10% of pregnant women will develop asymptomatic bacteriuria during pregnancy (7), and left untreated, one third of cases will develop a symptomatic UTI, with the potential to cause preterm prelabour rupture of membranes (PPROM), chorioamnionitis, pyelonephritis and preterm birth (PTB) (8). Together with antibiotic prophylaxis recommended for the management of PPROM, risk based intrapartum chemoprophylaxis to prevent Group B streptococcal early onset disease (GBSEOD) of the neonate, and antibiotic prophylaxis for caesarean section (CS), about 20-25% of pregnant women will be given at least one hospital prescribed antibiotic, mainly a penicillin, during pregnancy (3, 6, 9). According to a large population-based Registry Study of nearly 1,000,000 Danish women, around 40% took community-prescribed antibiotics at some stage during pregnancy (2), and the use of antibiotics was more likely in women who were young, obese, or of lower socioeconomic status. The study took no account of antibiotics administered as hospital in-patients, nor of self-administered antifungals, or antiviral agents bought over-the-counter (OTC). Accordingly, this is highly likely to be an underestimate. Recent concerns have been raised about the use of antibiotics in pregnancy, and the adverse effects these may have on the neonatal gut microbiome, particularly that of preterm infants (10, 11). One of these side effects involves alterations to the development of the infant immune system, leading to the development of atopy, asthma, allergy and obesity in childhood (12).

2.0 The human microbiome project

Following conclusion of the Human Genome Project, scientists were surprised to find only 23,000 protein coding genes, similar to that of the fruit fly (13). However, if one considers the sum of all human genes, plus the collective symbiotic microbial genome (microbiome), the human microbiome may include considerably more than 1,000,000 genes, supporting the concept that the human microbiome provides genetic functions that we have not needed to evolve for ourselves (14, 15). The core human microbiome, as well as the variable human microbiome, (influenced *inter alia* by genotype, environment, host lifestyle, pathophysiology, immune response, and transient community members), is being assessed spatially and temporally, and different sub-sites are also being analysed (13). While it may be possible to manipulate the human microbiome to influence physiology, the use of antibiotics at crucial stages of early development, may adversely affect that development.

2.1 *New information from molecular-based cultivation independent studies*

Molecular-based, culture-independent studies have demonstrated that only 20% of microbiota can be detected by traditional culture-based techniques, leading to previously unidentified organisms, or organisms that were under-detected and hence under-appreciated (16). This in turn has led to more information about the normal (eubiosis) and abnormal genital tract microbiota (dysbiosis), as well as the influence of pregnancy and delivery on the neonatal gut microbiome.

2.1.1 *Maternal genital tract microbiome*

2.1.1.1 *Vaginal eubiosis*

The healthy human vagina is colonised by populations of lactic acid producing bacteria, mainly of the genus *Lactobacilli*, that maintain the pH of the vagina below 4.5. At this acid pH, the growth of potentially pathogenic bacteria is suppressed. Using solely culture-dependent techniques, it is not possible to identify *Lactobacillus* spp beyond the genus level. That is to say, we know the organism is of the Genus *Lactobacillus*, but we cannot comment on the species, and hence cannot comment on species-specific functions such as H_2O_2 or lactic acid production, or production of natural antimicrobials such as bacteriocins. This being the case, with over 250 species of *Lactobacilli* currently identified, mostly used in the food industry, it is not surprising that 25 years ago, some concluded that, "no two women have the same vaginal lactobacilli" (17). Using molecular-based, cultivation-dependent techniques we now know that this assertion is wrong, and that worldwide, the eubiotic vaginal microbiota is colonised by one, or at the most two species of *Lactobacilli,* from a shortlist of four species: *Lactobacillus crispatus, Lactobacillus jensenii, Lactobacillus gasseri,* and *Lactobacillus iners* (16).

2.1.1.2 *Vaginal dysbiosis*

Vaginal dysbiosis may be manifest by: i) the introduction of an abnormal organism that is not part of normal vaginal microbiota, either by sexual transmission such as *Neisseria gonorrhoeae, Chlamydia trachomatis* or *Trichomonas vaginalis*, or non-sexually transmitted, by organisms such as *Haemophilus influenza;* ii) by an increase in the virulence of existing organisms such as *Esch. coli*, or iii) an imbalance of the normal microbiota, in which species of *Lactobacilli* are reduced in quality and quantity, the vaginal pH rises, and there is a 1000-fold increase in other organisms such as anaerobes, *Gardnerella vaginalis*, and species of *Mycoplasma* and *Mobiluncus.* This latter form of dysbiosis is called bacterial vaginosis (BV). New information from molecular-based, cultivation-independent techniques (16), has detected that BV is not a single entity, but composed of a number of subtypes (at least 2-4), composed of different bacterial communities (18). This would go a long way to explain the problems with BV with respect to the aetiology, microbiology, response to antibiotics, phenotypic outcome and treatment of the sexual partner.

2.1.2 *Influence of the neonatal gut microbiota on the immune system in infants*

The human gastrointestinal tract contains hundreds of microbial species that provide a multitude of functions, including the development of the host immune system. Intestinal dysbiosis has been shown to occur with the use of antibiotics in early infancy (19), and maternal use of antimicrobials in the antenatal or intrapartum period. This may alter the neonatal gut microbiota, leading to long-term effects on immunological maturation, and increasing the potential for chronic disease (20). Low diversity of bacteria has been found in neonates exposed to antibiotics *in utero,* particularly reduced levels of species of *Lactobacillus* and *Bifidobacterium*, the first microbes known to colonise the human gut in infancy, and suspected of initiating a vital immune ecosystem. When used at or after birth, antibiotics may disrupt mother-to-newborn transmission of the healthy microbiota of the maternal gut, vagina, skin and/or breast milk (21, 22). In addition to effects on the microbiome and metabolome, antibiotics may alter epigenetics (23) and fetal development (24). An important question is whether, through these mechanistic associations, maternofetal exposure to antibiotics increases the risk of immune-mediated diseases, such as asthma (22). Intrapartum antibiotics, as well as prolonged rupture of the membranes, independently lead to a reduction in transmission of lactobacilli to the neonate (25), and in babies exposed to antibiotics *in utero,* there is delayed colonisation of intestinal flora (26). With respect to the gut microbiota profiles of 198 infants, the use of intrapartum antimicrobials was associated with dysbiosis up to 1-year of age, involving a reduction in *Bacteriodetes,* and greater quantities of *Clostridium* and *Enterococcus* spp, frequently

found to be associated with low gut microbiota diversity in patients with atopic disease. The study suggested a degree of 'microbiota recovery' throughout the first year of life, which was more evident in exclusively breastfed infants (27).

The biological model used to explain this observation involves the disruption of developing gut microbiota, which results in a failure of maturation of the immune response (28). This has subsequently been demonstrated in animal studies, in which the maternal microbiota and metabolites *in utero,* may modify the fetal innate immune system and cause allergic airway disease (29, 30). The immune system is primed *in utero* and modified after birth. Factors that modify microbial exposure *in utero* may also influence the development of allergy (31). Antibiotics may induce a reduction and modification of the neonatal gut microbiome, which affects the developing immune system in a way that promotes allergic disease development. Epidemiological studies have demonstrated that the composition of the gut microflora in atopic children is different to normal controls (32).

2.2 *The hygiene hypothesis*

Atopic disease is a syndrome of hypersensitivity, which typically presents as one or more of an 'allergic triad' of symptoms: i) allergic asthma; ii) eczema (allergic dermatitis) and iii) hayfever (allergic rhinitis), and affects around 25% of the population in industrialised countries, especially children and young adults (33). This is associated with a large percentage of allergy-associated co-morbidities and creates a strain on healthcare systems. Atopy is caused by a mixture of genetic and environmental factors, with 60% monozygotic concordance (34). The prevalence of allergic disease in high-income countries has dramatically increased in recent decades. The "Hygiene Hypothesis", was first proposed by David Strachan after the observation (from 17,000 British children born in 1958), that there was an inverse correlation between hay fever and the number of older siblings (35). According to the hygiene hypothesis, this geographical and chronological trend is the manifestation of a decreased exposure of children to infections that have an important role in the development of a healthy immune system, and antibiotic use is thought to contribute to this effect. Antibiotic exposure in the first year of life increases the risk of allergic disease, so the use of antibiotics *in utero* may also adversely affect the developing immune system towards atopy and allergy (32). Some of the changes that antibiotics induce to the microbiota and its metabolite production, disappear within a few weeks or months of discontinuation, whereas others last much longer (36). Accordingly, antibiotics taken preconceptually may have residual effects during pregnancy, and increase the long-term risk of asthma in children (37). Similarly, maternal antibiotics taken postnatally may alter vertical transmission of microbiota in the maternal skin or breast milk (38). This being the case, such alternative explanations, do not support or refute a directly causal pregnancy-specific relationship. Intuitively, if one accepts the "hygiene hypothesis", a rural population with more exposure to diverse microbial communities would be more protected against the risk of asthma. A novel observation confirmed this theory, in which a rural population, though subject to a higher rate of antibiotic use, had a lower rate of asthma (39).

3.0 Methods

The databases of MEDLINE/Pubmed, Embase, and Cochrane Database of Systematic Reviews were searched using the following keywords and MeSH terms: "pregnant/pregnancy", "complications," "prenatal environment" [AND] "antimicrobial/anti-infective agent" [AND] "infant gastrointestinal tract/bifidobacterium/microbiota/microbiome" [AND] "hypersensitivity/atopic disease/asthma". Animal studies were excluded, English language restriction was added, and the search was supplemented by additional reports identified from the reference list of published articles. Our database search returned 2,911 results, and after exclusion of duplicates and screening of titles, 140 abstracts were screened, of which 102 were excluded, leaving 38 papers for full analysis. Of these 38 publications, 14 were considered irrelevant or applied to antibiotic use in childhood rather than

in pregnancy, so 24 papers were considered relevant, relating to the use of antimicrobials during pregnancy, the neonatal gut microbiome and the development of atopy, asthma and allergy in childhood. These were selected for use in the review (Figure 1).

4.0 Results

4.1 *Allergy, asthma and atopic disease*

4.1.1 *Historical evidence*

In 2011 and 2013, two systematic reviews evaluated antenatal exposure to antibiotics and the prevalence of different atopic conditions (32, 40). One included four studies comprising 26,276 cases, which overall showed no significant association between antenatal antibiotic exposure and eczema (32). However, three of the four studies (31, 41, 42), found a significantly positive association between antibiotic use in pregnancy and eczema, two of which (31, 41), also examined other atopic diseases, and found positive associations with hay fever and asthma. Only one of the four studies (43), failed to find an association, but antibiotic exposure was not one of the main variables, and it was unclear how many antibiotic-exposed infants were included in the results. In addition, the cohort was highly specific, and only included Caucasian mothers with a history of asthma. In a study not included in either systematic review, perhaps because it was a population-based cohort study, it was reported that early pregnancy infections were more likely to be associated with eczema when the mother was treated with broad-spectrum antibiotics (44).

The other systematic review (40), found three relevant studies, one of which (31) was also included in the other systematic review (32). An increased risk of asthma with borderline significance between exposure to antibiotics in pregnancy and childhood asthma was found. Both studies showed stronger evidence to support an association with antibiotic use in the first year of life and atopy, compared to antenatal exposure. A large study that was included in both systematic reviews involved nearly 30,000 births from a UK database, and found a significant association between antenatal exposure to antibiotics, with an increased risk of atopic disease. However, the association with atopy and antenatal exposure alone was only significant if three or more courses of antibiotics were administered (28). However, there were confounding factors in this study, since mothers with atopy were more likely to have children with atopy. These mothers were also more likely to need antibiotics during pregnancy, as they are more susceptible to chest infections in association with asthma or skin infections with eczema. Accordingly, the use of antibiotics may not be causative, but a consequence of an independently established increased risk. However, this hypothesis has subsequently been undermined, since correcting for maternal asthma did not change this association (39).

Since these two systematic reviews were published, we have found several other studies that were not included, and these as summarised in Table 1. While many studies have shown a strong association between antibiotics in early childhood and allergic disease in later life, more recent research has sought to answer the question of whether the mode of delivery *per se,* as well as antenatal and intrapartum antibiotics, have similar effects. Although not statistically significant, a population study of 723 mother-child pairs, found that by two years of age, children born by vaginal birth had a lower risk of developing eczema compared with those born by CS. This finding was stronger in those children whose mothers had not been given systemic antibiotics or antifungals during pregnancy (45).

4.2 *Asthma*

In the last 40 years, the global incidence of asthma has increased, with an estimated 334 million cases in 2014 (46, 47), and in children aged 5 to 14-years, asthma is the most common chronic disease (46). Though the aetiology of childhood asthma is multifactorial, maternal asthma, exposure

to cigarette smoke, and air pollution in pregnancy, are among the risk factors for asthma in offspring (48, 49), together with PTB and low birth weight (50). Recently, it has become evident that perturbations of microbial communities, and the metabolites they produce (36), may affect the normal mother-to-newborn transfer of microbiota, and play an important role in the pathogenesis of asthma. An increasing number of studies have demonstrated a positive association between the use of antibiotics during pregnancy and subsequent childhood asthma, even after controlling for confounding factors (51-56). However, two large Scandinavian registry based studies (55, 56) among these, suggested that the association may be due to a maternal propensity for infection rather than the use of antibiotics *per se*. To support this, one study used sibling analysis (55), and the other (56) pointed out that the use of maternal antibiotics was not specific to pregnancy, but was also observed when used before conception and postpartum. The association was also stronger for antibiotics prescribed for respiratory infections. A recent large population-based cohort study of mother–child pairs in Canada (39), supported the contention that the association was not unique to use during pregnancy. However, they provided fresh evidence that maternal antibiotic exposure is associated with a dose-dependent increase in the risk of childhood asthma. Adjusting for infant gender, method of infant feeding, and mode of delivery, most classes of antibiotics were associated with a risk of asthma, though the degree of risk varied.

4.3. *Role of vitamin D*

Vitamin D is an immuno-modulator that is strongly associated with a number of pregnancy outcomes (57), and extra-skeletal outcomes in children (58). In pregnant minority adolescents at particular risk of vitamin D deficiency because of poor diet, dark skin (at northern latitudes), and urban living, the rates of BV in pregnancy, which is strongly associated with PTB (59), is directly related to serum levels of vitamin D (60, 61). Hypovitaminosis D is associated with a higher rate of infection in pregnant women (62) and asthma in their offspring (63). In addition, supplementation with vitamin D reduces recurrent wheeze and childhood asthma, as well as the risk of infection and the need to use antibiotics in pregnancy (64).

4.4 *Lactose intolerance*

Cow's milk allergy accounts for around 2-6% of food allergies in the first year of life (65) and presents a strain on healthcare in general, and paediatric services in particular. The reasons for the development of food intolerance are thought to be a mixture of genetic and environmental factors. However, more recent studies have addressed modifiable risk factors, and whether antimicrobials in pregnancy can have an effect. We found two studies that addressed the question of lactose intolerance and the use of antibiotics in pregnancy. A population study of 16,237 reported that the use of antibiotics before or during pregnancy, led to an increased risk of cow's milk allergy in offspring (66). A retrospective cohort study found similar results, and reported that children who were breastfed for longer, had reduced rates of cow's milk allergy. The study found no association with gestational age or mode of delivery, but had a small sample size of only 101 children (67).

4.5 *Atopic dermatitis*

We found two studies (68, 69) that examined the use of prenatal or intrapartum antibiotics and the subsequent development of atopic dermatitis in childhood, the characteristics of which are shown in Table 2. The first of these examined 492 children delivered vaginally. Of those exposed to antibiotics, exposure for >24 hours increased the subsequent risk of atopic dermatitis, whereas <24 hours exposure to antimicrobials did not (68). In a Korean birth cohort study, CS with antenatal antibiotic exposure was significantly associated with atopic dermatitis in infancy (69).

4.6 *Obesity*

In less than one generation, the worldwide prevalence of overweight and obese children has risen

substantially (70). The human gut microbiome plays an important role in adipogenesis and any alteration in the gut microbiome increases the susceptibility to obesity in later life (71). We found five studies that addressed the role of antibiotic use in pregnancy and subsequent childhood obesity (24, 72-75), and the characteristics of these studies are shown in Table 3. In a large study of 9886 children in Denmark, those that had been exposed to antibiotics antenatally, had a 26-29% rise in the prevalence of obesity at school age (up to 16 years of age). However, a limitation of the study was the lack of information about maternal obesity, which is known to lead to a higher propensity for childhood obesity (72). In another study, antibiotics administered in the 2nd or 3rd trimester, led to an 84% greater risk of obesity by the age of 7-years when compared with children who were not exposed to antenatal antibiotics. There was also a statistically significant risk of a higher body mass index (BMI)-Z scores, (a measure of relative weight adjusted for child age and sex), waist circumference, and body fat percentage in children who received antibiotics *in utero*. Of those children delivered by CS compared to those who had a vaginal birth, 46% had a greater risk of obesity, regardless of whether the CS was elective or emergency (73). To compare antimicrobials with antifungals for any association with obesity in childhood, a cohort of 527 children was studied. In the cohort, 57% had been exposed to antibiotics *in utero*, and 19% to antifungals. Antibiotics but not antifungals were associated with a raised BMI Z-score by the age of 2-years. The association was strongest when antibiotics were given in the 1st and 2nd trimester. However, pregnancy dating relied on accurate recall of the last menstrual period to assign treatments to specific trimesters and this was considered a limitation of the study (74). A more recent observational study of 2128 women, measured umbilical cord leptin levels as a marker of fetal adiposity. In a trimester-specific analysis, antibiotic therapy in the 3rd trimester was associated with raised cord leptin levels, but this was not the case when considered over the whole of pregnancy (24). There were several limitations to this study, including no reason given for antibiotic therapy, no record of adherence, and no adjustment for possible confounding factors. There remains a mixed opinion on whether antibiotic usage is associated with alterations in fetal adipokines. Only one study found no association between antenatal antibiotics and childhood obesity. Initially, a strong association between childhood antibiotic exposure and raised BMI was found, and also that mothers who had received ≥3-courses of antibiotics in pregnancy were more likely to have children with a high BMI. However, once adjusted for co-variants, this association was no longer significant (75).

In addressing the role of antibiotic use in pregnancy and subsequent childhood obesity, we accept that the evidence is new and sparse, we also accept that obesity is a complex phenotype that is strongly affected by maternal nutrition and health before and during pregnancy. There are also other confounding factors, not fully covered in the studies cited, such as maternal diet during pregnancy, gestational weight gain and its timing, and gestational diabetes.

5.0 **Interventions**

5.1 ***Seeding therapy***

Vaginal seeding, or microbirthing (first proposed by in 2016), involves the postnatal transfer of maternal vaginal fluid to a neonate born by CS, aiming to correct the partial disruption that occurs to the newborn's microbiota following CS compared with vaginal birth (76). Vaginal seeding has attracted much interest amongst the public and professionals with increasing numbers of mothers taking up the practice, while practitioners raise concerns about its safety. Few studies have been carried out to provide confidence that vaginal seeding is safe in humans (77). The potential for transfer of asymptomatic vaginal pathogens to the newborn, such as *C. trachomatis*, *Herpes Simplex Virus* or *N. gonorrhoeae* remains a risk. In the UK, intrapartum antibiotic prophylaxis for the prevention of GBSEOD of the neonate is based on risk rather than screening during pregnancy (78), so there is genuine concern that this significant cause of neonatal sepsis could be transferred inadvertently to the newborn through vaginal seeding. In the absence of evidence to prove that

vaginal seeding is beneficial, healthcare professionals in the UK have been advised not to undertake vaginal seeding, and to inform women of the potential risks if they intend to pursue this route independently.

In 2017, the vast majority of Obstetricians in the Danish Society of Obstetrics and Gynaecology (DSOG) chose not to recommend vaginal seeding in relation to CS. They recommended that maternity wards should not participate: i) in guidance on how to perform vaginal seeding; ii) in the screening of the women prior to vaginal seeding, and iii) actively in the procedure for vaginal seeding. They also advised strongly against vaginal seeding in the following situations: i) mothers who meet national criteria for GBSEOD prophylaxis during delivery; ii) gestational age less than 37 completed weeks at delivery; iii) in clinical situations where CS is recommended to prevent perinatal infection during vaginal birth such as primary vaginal HSV, and HIV infection with a heavy viral load, and iv) other clinical situations where vaginal seeding was considered to be potentially harmful to the neonate, such as certain fetal malformations. They went on to say that, parents who personally wish to undertake vaginal seeding: i) can perform the procedure provided it does not interfere with, or delay other procedures and ii) should be informed by their physician about the lack of evidence, and the potential risk. Furthermore, they should be provided with information (provided by the DSOG) and must be advised to be vigilant with respect to signs of neonatal disease and to indicate that they have performed vaginal seeding if the neonate requires medical attention. The DSOG guidelines concluded that the recommendations applied to clinical management and did not exclude the performance of vaginal seeding as part of a clinical trial with proper research board supervision (79). For further debate on this subject, the reader is referred to the excellent commentary (80) that accompanied the DSOG recommendations and the subsequent correspondence (81, 82), which concluded that, overall, with respect to vaginal seeding, there remained more questions than answers. Neonatologists have offered alternatives such a promoting breastfeeding as well as the introduction of probiotics. Hopefully, new information about the species-specific role of *Lactobacilli* from the vaginal microbiome project (16) will provide additional information for the development of better probiotics (83, 84).

5.2 *Neomune Project*

Developed by the University of Copenhagen, the Neomune Project aims to study the ways in which both early microbial colonisation as well as diet, can have an impact on the development of the neonatal immune system and cognitive function. The more vulnerable of these infants are those born preterm, or small for gestational age, a cohort accounting for around 15% of newborns. So far, research has not clearly identified how best to support the needs of such infants, and the Neomune Project aims to investigate microbial and dietary elements that aid maturation of the immune system. With this, it is hoped that there will be more accurate development of formula milk to support infants for whom breastfeeding cannot occur, or is contraindicated, while contributing to the currently limited research into the addition of probiotics to milk products. Early results suggest that these probiotic supplements have the potential to support the development of the gut immune system, while simultaneously suppressing pathogens in vulnerable newborns. However, more information is required before widespread use can be recommended.

(see: https://food.ku.dk/english/research_at_food/research-projects/2014/neomune/).

5.3 *Probiotics and breastfeeding to correct microbial disruption in infants exposed to in utero antibiotics or delivered by caesarean section*

As the first food type to be introduced to the infant gut following birth, milk is believed to have the potential to shape the developing microbiota, and similarities between the bacterial make-up of maternal colostrum and newborn meconium have been demonstrated in babies breastfed within

the first hour of life (85). *Bifidobacterium*, is dominant in human breast milk, and has been shown to protect the infant gut from pathogens, and leads to overall health benefits in later life. This occurs through transfer of glycosylated proteins present in breast milk. However, levels of these bacteria are reduced in the breast milk of women who deliver preterm, as well in those delivered by CS, although this could be related to the prophylactic antibiotics given to cover surgery, and this needs clarification (86). As a result, Professional bodies such as the Royal College of Paediatrics and Child Health, as well as the American Academy of Pediatrics, highlight the importance of breastfeeding as a public health concern, and advocate the use of donor breast milk, where maternal feeding is contraindicated, or not possible, rather than resorting to the option of formula feeds (87).

There is also increased interest and research into the use of probiotics. The WHO defines probiotics as, "live organisms, which when administered in adequate amounts, confer a health benefit on the host". Probiotics are reported to balance the intestinal flora, which has led to increased consumer interest in such products. However, reliable information remains lacking with respect to safety in the context of materno-fetal and neonatal effects, and some studies are reporting mixed findings. In neonatal mice, T-cell disturbance secondary to antibiotic use was prevented by probiotic administration (88), and potential improvements in mucosal tolerance following probiotic administration have been reported (89). More recent studies have found that probiotics may lead to a reduction in the incidence of PTB as well as allergies and asthma in children. In contrast, higher levels of *Bifidobacterium* in probiotic treated infants lasted for one week only, with no difference thereafter, and was associated with increased infections in later life (90). Accordingly, it must be concluded that gaps remain in our understanding of probiotics, and further well-conducted research is required.

6.0 Conclusion

In conclusion, there is growing evidence to support an association between antibiotic exposure *in utero* and atopic disease in the form of asthma and eczema and also childhood obesity. The contribution of CS rather than vaginal birth is also recognised. More research is needed, and such studies must be careful to avoid common confounding variables (91). A more robust approach to addressing this question would be to use secondary analysis of previous randomised trials of prenatal antibiotics (59) with long-term follow up, addressing the incidence of obesity, asthma, eczyma and other atopic disease.

Healthcare professionals are faced with the dilemma of, on the one hand, pressure to reduce the use of antibiotics because of the problems of resistance (12), and at the same time being under pressure to implement the early use of antibiotics to reduce the serious mortality and morbidity associated with sepsis (92). For various reasons, a significant percentage of pregnant women receive antibiotics in pregnancy though few studies exist to quantify this use. Accordingly, it is important to consider the long-term use of this practice. From our review of the literature, there is sufficient information to raise concerns that the use of antibiotics in pregnancy has the potential to influence the future development in childhood of asthma, allergy, atopy and obesity. Up to 30% of prescriptions for antibiotic can be unnecessary (93) so guidelines have been produced to prevent such use. In following guidelines for any intervention in pregnancy, obstetricians have difficulties that are not experienced by colleagues in other specialities. This is because, in obstetrics, there are two patients to consider (mother and fetus) and while a course of antibiotics may be helpful for the mother in the short term, there may be long-term adverse fetal effects such as birth defects (94) and other child health outcomes like obesity, asthma and atopic disease (12).

7.0 Expert opinion

7.1 Interesting areas of research

The “Barker Hypothesis” in which our health as adults, and the quality of life of future generations, may be determined by care or complications during our fetal or early neonatal life, is now well recognised. The realisation of this hypothesis is manifest in the association between intrauterine malnutrition and under-nutrition during fetal or early postnatal life with chronic hypertension, diabetes, stroke, and death from cardiovascular disease (95). The pertinence of this hypothesis, as it pertains to perinatal medicine, is comprehensively covered in the editorial by Roberto Romero in, “The Child is the Father of the Man” (96).

At roughly the same time as the Barker Hypothesis was proposed (late 1980s), David Strachan proposed the “Hygiene Hypothesis”, and the two complement each other. Both hypotheses are remarkably intuitive, and beg the question, “why didn't we think of this before?” The hypotheses are also supported by recent evidence about the aetiology of acute lymphoblastic leukaemia (ALL), a disease of affluence, the commonest cause of childhood leukaemias, and the major cause of childhood cancer in high-income countries (97). The incidence of ALL is rising by 1% each year in high-income countries, and is more common in 1st born infants. While it could be argued that today, children are exposed to infection through new vaccination programmes to stimulate an immune response, the antigens used to develop such vaccines are often based upon attenuated viruses (98), or in the case of Group B haemolytic streptococcus, virulence factors such as capsular polysaccharides (78), rather than wild organism that may be more antigenic in stimulating an immune response.

The theory behind this finding in ALL is based on a gene-environmental interaction in two stages. The first stage occurs *in utero* with an accidental genetic change. The second stage makes the immune system more prone to react to infection after birth, but only in children brought up in a relatively germ-free environment. As Hawkes says in his commentary, “the first change loads the gun, the second pulls the trigger” (97). In children raised in such a sanitised environments, without siblings, with little or no social contact before the age of one year, the process of development of the immune system is faulty. This fault will be less evident in children born by vaginal birth and those that are breastfed. The most primitive of threats to organisms of all taxonomic orders is, i) threat from a predator and ii) threat from lack of nutrients (99). Accordingly, the immune system evolves in an environment of infections, so infection is needed for it to form correctly (100). The paradox is, that the development of ALL may be because there has been a lack of exposure of the immune system to antigens in infections, yet the adverse response from the accidental genetic change may be triggered by infection. The absence of rough-and-tumble in the first year of life leaves the immune system unable to respond appropriately at a later date.

7.2 Key findings and weaknesses.

The key finding of this review is the increasing evidence for the role of antibiotic use in pregnancy, and subsequent childhood development of obesity, asthma, eczyma and other atopic disease. The weakness of the research is that, particularly in earlier studies, insufficient assessment was paid to confounding variables. There remains uncertainty as to whether or not it is relevant that the mother suffered from the same condition (eg asthma) as the purported infant outcome variable, for which she may have received antibiotics. In most studies, there is also a lack of control for the number of antibiotic courses administered, the timing of use, the use of broad spectrum or narrow range antibiotics, the indication for antibiotic use, the dose dependent nature of the effect, the class of antibiotics used, or a varying degree of risk.

7.3 Ultimate goal for this research.

The ultimate goal for this research is to reduce the increasing rate of obesity, asthma and atopic disease in children, that leads to impaired quality of life in adulthood, and in future generations, manifest *inter alia* by chronic hypertension, diabetes, stroke, and death from cardiovascular disease.

7.4 What research or knowledge is needed to achieve this goal and what is the biggest challenge.

The association appears to be aggravated by CS and artificial feeding rather than the putative benefits of vaginal birth and breast-feeding, and this relationship needs to be further investigated. In addition, since there is increasing realisation that the effect of antibiotics on the maternal microbiome may be long lasting, the effect of pre-pregnancy antibiotics as well as antibiotics in pregnancy needs to be addressed in future studies. It needs to be established whether the effect of antibiotics on the infant is causative or as a consequence of an independent increased risk. The risk of infant atopic disease has to be assessed in the light of maternal disease, whether or not there is a pre-existing genetic predisposition to a condition like asthma or whether it is because of the likelihood of maternal use of antibiotics that constitutes the risk factor for subsequent atopic disease. In addition, future studies will need to correct for this variable and also for mode of delivery and infant feeding, bearing in mind the increased risk associated with CS and artificial feeding as opposed to vaginal birth and breast-feeding.

With respect to CS *per se*, there is still debate about the use of prophylactic antibiotics. In most units, prophylactic antibiotics are given with CS but this is a mainly narrow-range antibiotic given after cord-clamping, the choice being driven by fetal indications (101). However, there is increasing evidence to suggest a change to broad-spectrum antibiotics given before incision, since this appears to provide better maternal protection, with no disadvantage to the neonate. However, this disadvantage has only been measured in the short term for concerns over unnecessary septic screens (101) and should now be tested for long term problems such as atopic disease.

7.5 What should happen in the coming years?

While the evidence for a link between maternal use of antibiotics before and during pregnancy is not undeniable, there is increasingly robust evidence to support the premise. However future research to increase the robust nature of the evidence should make adjustments for confounding variable such as those listed above. Efforts should be made to reduce unnecessary use of antibiotics in general, but particularly in pregnancy. Much of the work presented, has occurred in high-income countries, mostly in non-tropical settings, so there is still significantly more to understand from low-income countries where infection patterns differ. This is especially true for parasitic diseases such as malaria and liver flukes, and for soil-transmitted helminths such as hookworm or ascaris, which also affect immunity and allergy, and have very different epidemiological pictures when present. More work in these types of setting is needed in future.

7.5.1 Overall use of antibiotics in pregnancy

Most obstetric units in the UK are unaware of the extent to which antibiotics are used in pregnant women booked for confinement under their care. Using registry data, around 40% took community-prescribed antibiotics at some stage during pregnancy (2). However this study took no account of antibiotics administered as hospital in-patients, nor of self-administered antifungals or antiviral agents bought OTC, so is likely to be an underestimate. It should not be difficult for obstetric units to audit prospectively the use of all antimicrobials during pregnancy, either prescribed by hospital or GP, or self administered as OTC purchases, as a basis to influence reduction in antimicrobial use.

7.5.2 Antibiotics for the prevention of GBSEOD infection of the neonate

Currently, the UK has a programme for the use of intrapartum antibiotic prophylaxis, for the prevention of GBSEOD of the neonate that is based on risk rather than a screening programme, which is the practice in the USA and elsewhere. Because of the cost of screening, the risk-based approach costs less (financially) than screen-and-treat, but is associated with a much higher use of antibiotics. Due to differences in the burden of disease, it is unlikely the UK will change from the risk-based approach to screen-and-treat. However, there are other measures that can be introduced to reduce the use of antibiotics for the prevention of GBSEOD of the neonate, such as vaccination and point-of-contact molecular diagnostic techniques (78).

7.5.3 Antibiotics for the prevention of PTB of infectious aetiology

Currently, there is controversy about the use of antibiotics for the prevention of infection-related PTB, particularly the choice of agent, the choice of patient and the timing of administration. Rather than an unfocussed approach to the prevention of PTB which results in the overuse of antibiotics, greater emphasis should be placed on a more focussed use of appropriate antibiotics (probably clindamycin), used early in pregnancy (before 22 completed weeks of gestation) in women with objective evidence of risk of infection-related PTB (mainly vaginal dysbiosis in the form of BV) (59, 102, 103) which should result in reduced use.

7.5.4 Long-term follow up using previous antibiotic trials.

Prospective, long-term follow up studies on the use of antibiotics in pregnancy, as well as prenatally and neonatally are necessary. In the meantime, secondary analysis of existing data from previous studies of antibiotic use in pregnancy (104-107), an increasingly popular method of enhancing health research (108), may provide important information. However, this depends on governments, funding agencies, and researchers making the data collected in primary research studies, and health-related registry systems, available to researchers who were not involved in the original study.

Funding
This paper was not funded

Declaration of interest
The authors have no 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. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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

Author contributions
RF Lamont accepted the commission, proposed a framework for the manuscript and provided guidance for the search strategy. S Milliken carried out the literature search, extracted the data, synthesised the evidence and provided the first draft of the manuscript. RF Lamont wrote the Expert Opinion Section and all three co-authors contributed to sequential drafts and the final version.

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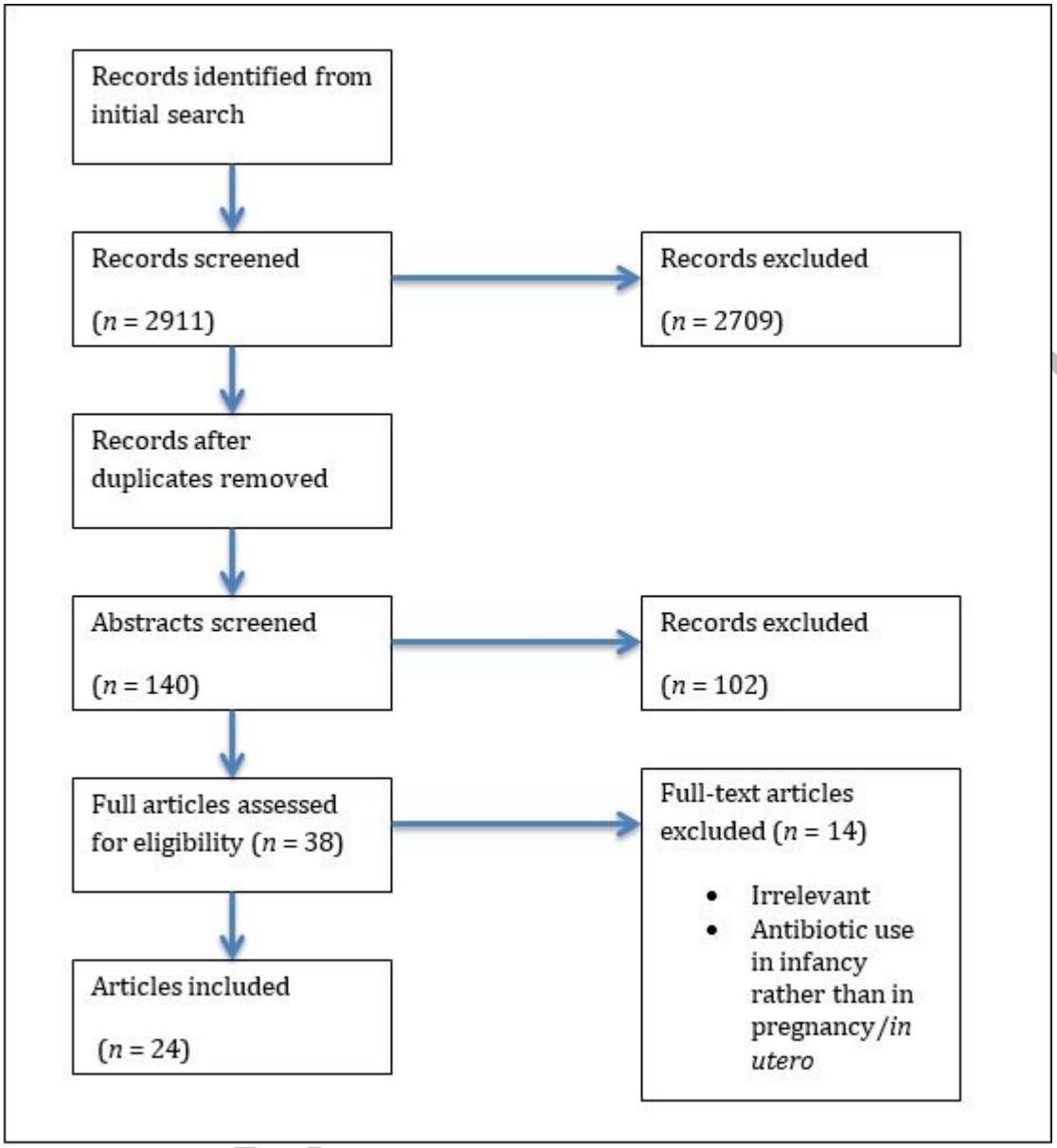
Table and figure legends

Table 1. Characteristics of Studies on Antibiotics During Pregnancy and Allergy, Asthma and Atopic Disease not Included in Two Systematic Reviews (32, 40)

Table 2. Characteristics of Studies on the Use of Prenatal or Intrapartum Antibiotics and the Subsequent Development of Atopic Dermatitis in Childhood.

Table 3. Characteristics of Studies on the Role of Antibiotic Use in Pregnancy and Subsequent Childhood Obesity.

Figure 1. Flow of Search



AUTHOR	YEAR	COUNTRY	POPULATION	TYPE OF STUDY	SUMMARY
Rusconi et al. (109)	2007	Italy	15,609 children (3278 'wheezers', 12331 'non-wheezers')	Retrospective Cohort Study	Maternal complications in pregnancy & birth associated with wheeze development in children
Martel et al. (110)	2008	Canada	Initial cohort 26,265 pregnancies. 1578 children ultimately analysed (745 asthmatic, 833 controls)	Case-control study	Identification of 16 most influential determinants of childhood asthma from 47 risk factors
Dominguez-Bello et al. (111)	2010	South America	9 women + 10 newborns (4 born vaginally, 6 born by caesarean section)	Cohort study	Microbiota of infants born vaginally similar to mother's vaginal microbiota. Those born by caesarean section shared similar microbiota to the mothers skin.
Schmitt et al	2010	Germany	2916 infants recruited at birth	National Birth	Infants with eczema have

(112)				Cohort Study	higher risk of mental health problems by age 10 years
Dubakiene et al.(113)	2012	Lithuania	128 infants (43 with food allergy, 85 controls)	National Birth Cohort Study	Infants of atopic mothers more at risk of egg intolerance than those of non-atopic mothers
Stensballe et al. (54)	2013	Denmark	411 infants (born to mothers with asthma)	Cohort study using National Registry Data N = 30 675	Increased risk of childhood asthma in those exposed to in utero antibiotics
Metsala et al (66)	2013	Finland	15,672 infants (diagnosed with cows milk allergy after 1 month of age)	Case control cohort study using National Registry Data	Maternal and childhood use of antibiotics associated with greater risk of cow's milk allergy in infant
Lee et al (69)	2014	Korea	412 infants (assessed at 1year of age for atopic dermatitis)	Cohort study	Both caesarean delivery and antibiotic exposure in utero may alter gut microbiota and subsequently affect risk of atopic dermatitis
Wegienka et al (114)	2015	USA	707 infants (enrolled in WHEALS)	Prospective Cohort Study	Children with higher early-life vitamin D levels have fewer allergy- related conditions
Toro et al (67)	2015	Mexico	101 infants (all diagnosed with cow's milk protein allergy(CMPA))	Retrospective, comparative, cross-sectional observational study	Breastfeeding duration in months and use of antimicrobials in pregnancy associated with CMPA
Wohl et al (68)	2015	USA	492 mother-child pairs (identified from birth records)	Retrospective cohort	Exposure to antibiotics for <24hrs during vaginal delivery shows no association with risk of developing atopic dermatitis

Table 1. Characteristics of Studies on Antibiotics During Pregnancy and Allergy, Asthma and Atopic Disease not Included in Two Systematic Reviews (32, 40)

AUTHOR	YEAR	COUNTRY	POPULATION	TYPE OF STUDY	SUMMARY
Lee et al (69)	2014	Korea	412 infants (9.9% exposed to antenatal antibiotics, 32.5% delivered by caesarean section)	National Birth Cohort Study	Increased risk of atopic dermatitis in children exposed to antenatal antibiotics and delivered by caesarean section
Wohl et al (68)	2015	USA	492 mother-child pairs (128 received intrapartum antibiotics)	Retrospective cohort using hospital records	Intrapartum antibiotic exposure >24 hrs may increase atopic dermatitis risk before age 2 years

Table 2. Characteristics of Studies on the Use of Prenatal or Intrapartum Antibiotics and the Subsequent Development of Atopic Dermatitis in Childhood.

AUTHOR	YEAR	COUNTRY	POPULATION	TYPE OF STUDY	SUMMARY
Mor et al. (72)	2015	Denmark	9886 children (3280 exposed to antenatal antibiotics)	Cross-sectional study	Exposure to antenatal antibiotics led to 26-29% increased risk of obesity at school age in these children, independent of birth weight.
Mueller et al. (73)	2015	USA	436 mother-child pairs (healthy, non smoking mothers recruited antenatally and followed for 7 years)	Population-based study	84% greater risk of obesity by age 7 years in children exposed to antenatal antibiotics in 2nd/3rd trimester than those not exposed. Children delivered by caesarean section (elective or emergency) 46% greater risk of obesity than vaginally delivered children.
Cassidy-Bushrow et al. (74)	2017	USA	527 children (303 exposed to antenatal antibiotics, 101 exposed to antenatal antifungals)	Prospective Birth Cohort	Antenatal antibiotic exposure but not antifungal use associated with raised BMI by 2 years of age
Mueller et al. (24)	2017	USA	2128 mother-child pairs (643 prescribed antibiotic during pregnancy)	Prospective Birth Cohort	Antibiotics in 2nd trimester associated with lower birth weight for gestational age. Antibiotics in 3rd trimester associated with higher cord blood leptin levels – associated with body weight status age 3 years
Poulsen et al. (75)	2017	USA	8793 mother-child pairs (5314 prescribed antibiotics during pregnancy)	Retrospective cohort study	Children whose mothers had ≥ 3 courses antenatal antibiotics at age 3 years – no longer present after adjusting for co-variates. Significant positive association between lifetime childhood antibiotic use and BMI.

Table 3. Characteristics of Studies on the Role of Antibiotic Use in Pregnancy and Subsequent Childhood Obesity