

Early-life antibiotic exposure, gut microbiome, and colonization resistance

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ABSTRACT

Antibiotics are chemical compounds that inhibit the growth of microorganisms to fight bacterial infections and, therefore, have been a central pillar of modern medicine. In recent years, there has been increasing awareness of the rising rate of their global consumption, especially in younger age groups. Exposure to antibiotics could happen not only during the first few months of newborns, but also indirectly through maternal consumption during pregnancy as well as during delivery. Indeed, epidemiological findings and meta-analyses have confirmed the association between early antibiotic exposures and risks for allergic diseases and obesity. Recent gut microbiome studies have provided mechanistic evidence supporting the role of antibiotic-induced dysbiotic gut microbiota in developing those diseases. However, no mechanistic evidence is available to convince restorative powers of breastfeeding on the disrupted gut microbiome and intestinal barrier function following antibiotic exposures in early-life. To address the fundamental questions related to complex traits of both exposure to antibiotic and breastfeeding's impacts in early-life, new experimental models are needed to differentiate short- and long-term effects of prenatal vs early postnatal exposure to antibiotics on gut microbiome and resistome (i.e., the collection of antibiotic resistance genes) of newborns.

Keywords: *gut microbiota, antibiotic exposure*

Introduction

Antibiotics, anti-infective agents, first discovered in the early twentieth century, have been widely applied in modern medicine and their global consumption has increased markedly over time [1]. In recent years, there has been increasing awareness of the rising rate of their global consumption, especially in younger age groups [2]. Antibiotics are commonly prescribed to children during respiratory tract and urinary tract infections [3-5]. Penicillins, cephalosporins, and macrolides are the antibiotic compounds commonly dispensed to children [6]. A study reported that broad-spectrum antibiotics, especially third-generation cephalosporins, are frequently prescribed for children in Eastern and Southern Europe, Asia, North America, and Latin America [7]. Another overlooked source of early-life antibiotic exposure is their administration to the mother during pregnancy or at birth [8]. Antibiotic exposure in early-life has been associated with later development of allergic conditions and obesity in childhood with positive associations confirmed by meta-analyses [9-11], although published evidence is not entirely consistent.

In recent years, gut microbiome studies have provided mechanistic evidence supporting the role of antibiotic-induced dysregulation of gut microbiota (termed dysbiosis) in developing those diseases [12]. An additional concern is the potential emergence of antibiotic resistance in the gut microbiome of newborns, following exposures to antibiotics in early-life [13]. Pregnancy is a critical period usually characterized by concurrent changes of endocrine, metabolic and immune functions intended to support the normal growth and development of the fetus [14]. During pregnancy, the maternal microbiota mainly vaginal and intestinal microbiota undergo drastic changes [15]. Maternal antibiotic use during pregnancy is known to affect the vaginal microbiota and, consequently, could hamper the vertical transfer of microbes to newborns during delivery [16]. Maternal microbiota during gestation plays a significant role in programming the future immune system of the offspring [17]. Although WHO and UNICEF have established recommendations to exclusively breastfeed newborns until their 6-month of age [18, 19], the decision of every mother is mainly influenced by economic, environmental, social, and political factors.

Current challenges and future directions

To address the fundamental questions related to complex traits of both exposure to antibiotic and breastfeeding's impacts in early-life, new experimental models are needed to differentiate short- and long-term effects of prenatal vs early postnatal exposure to antibiotics on gut microbiome and resistome (i.e., the collection of antibiotic resistance genes) of newborns. This knowledge will fill current gaps and contribute to develop a framework for antibiotic stewardship in perinatal and neonatal care. Importantly, breastfeeding itself and/or microbial biomarkers related to breastfeeding are of potential future interventions and policy implementation in infants in order to maintain a healthy gut microbiota development. We posit that microbiome sequencing efforts will contribute to potential selection of biomarkers that may be used in future interventions and will contribute to mechanistic understanding by highlighting potential mechanisms of how maternal breast milk restores gut microbiota and intestinal barrier functions in newborns under the pressure of antibiotics.

In addition to birth mode and neonatal diet, others and we have confirmed that early-life exposure to antibiotic has a profound impact on the composition and functions of the gut microbiota [20-22]. Animal experimental studies focused in early-life are providing a growing body of evidence that early antibiotic exposure leads to gut dysbiosis, and subsequent impairments in metabolic and immune functions as well as behavior [23-32]. Several human studies have reported a reduced abundance of *Bifidobacteria* and an increased *Enterobacteriaceae* in neonates who exposed antibiotics in early-life [20, 33-35]. Among them, some studies have indicated that the dysbiotic gut microbiota cannot fully recover months after cessation of antibiotic treatment. Furthermore, a single dose of antibiotics during birth, known as intrapartum antibiotic prophylaxis (IAP), often used in caesarean section, can significantly alter the infant gut microbiota and this effect has been shown to persist until at least three months of age [20].

A serious consequence of antibiotic-induced alternations in gut microbiome is the emergence of resistome and its links to colonization resistance against multidrug-resistant bacteria [36]. Surprisingly, limited data are available on the impact of early-life antibiotics in the development of resistome by the gut microbiota. Antimicrobial resistance is an important concern for global public health authorities. The enrichment in the resistome may increase the risk for transfer of resistance genes towards potential pathogens, further compromising the clinical management of infections. Some studies have demonstrated the existence of resistance genes in the early-life microbiota [37, 38]. A recent report demonstrated the enrichment of genes conferring resistance to β -lactam antibiotics together with increased *Proteobacteria* and *Firmicutes* following

maternal IAP [39]. The association between maternal IAP and expression of β -lactam resistant genes in the oral microbiome of both mother and neonates has been previously reported [40]. Well-established findings also indicate that gut microbiota plays a crucial role in the host's defense against pathogens such as *Clostridium difficile* [41, 42]. This can be a concern particularly relevant to hospital settings where antibiotic use and antibiotic-resistant exposure are more frequent. Antibiotic-induced perturbations in gut microbiome disrupt bacterial metabolic functions and colonization resistance against *C. difficile* but this appears to be dependent on antibiotic type [43]. In contrast, there is limited data available to link antibiotic-induced gut microbiome changes with subsequent colonization resistance against multidrug-resistant (MDR) *Escherichia coli*. The emergence of MDR *E. coli* is a fast-rising concern in children, due to its high prevalence mainly in resource-limited countries, where contaminated environment might be the source of infection [44].

Studies in animal models have shown that antibiotic exposure during pregnancy provides a dysbiotic microbiota with a reduced diversity in the gut of offspring [28]. Furthermore, antibiotic-induced gut dysbiosis can lead to a disruption in colonization resistance against bacterial pathogens [45-47]. Several efforts, therefore, have been undertaken to investigate how to restore altered gut microbiome. Although fecal microbiota transplant (FMT) is a promising approach to restore disrupted gut microbiome in adults, there are challenges to apply it in younger children. In this context, scores of studies have confirmed that breastfeeding is a natural remedy to restore beneficial microbes of early microbiome in children and protect against negative health outcomes later in life [48, 49]. However, no mechanistic evidence is available to support the restorative powers of breastfeeding on the disrupted gut microbiome and intestinal barrier function following antibiotic exposures in early-life.

Breast milk, a natural diet of newborns, contains a diverse population of microbe that may colonize the infant gut through breastfeeding. Colonization of the infant's gut is a complex trait depending on multiple factors including gestational age, birth mode, type of feeding, and environmental exposures [50]. Multiple studies have reported differences in gut microbiota profile of breastfed vs non-breastfed individuals during infancy as well as adulthood [51]. Our recent study confirmed that breastfeeding has a beneficial impact on early gut microbiota development and subsequent protection against childhood overweight [52]. Another study also showed that breastfeeding is highly associated with reduced abundances of specific antibiotic resistant genes and mobile genetic elements through modulation of microbial community in the gut [53]. Furthermore, breastfeeding has been known to provide higher colonization resistance against *Enterobacteriaceae*

compared to the formula-feeding (54). Notwithstanding these few and far between reports, data on the role of breastfeeding in the context of antibiotic-induced gut dysbiosis is largely missing.

We prone a stepwise approach to dissect in mechanistic terms how breastfeeding provides restorative effects on antibiotic-induced gut dysbiosis in early-life and subsequent colonization resistance. Our preliminary data point to a beneficial effect of breastfeeding, which restores the abundance of a probiotic bacterium whose abundance was diminished by early antibiotic exposure. We are confident that this strategy will generate translational findings that could also be applicable in implementing and developing policies and guidelines in pediatric care and management.

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