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Antibiotics and the developing infant gut microbiota and resistome

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The microbial communities colonizing the human gut are tremendously diverse and highly personal. The composition and function of the microbiota play important roles in human health and disease, and considerable research has focused on understanding the ecological forces shaping these communities. While it is clear that factors such as diet, genotype of the host, and environment influence the adult gut microbiota community composition, recent work has emphasized the importance of early-life assembly dynamics in both the immediate and long-term personalized nature of the gut microbiota. While the mature adult gut microbiota is believed to be relatively stable, the developing infant gut microbiota (IGM) is highly dynamic and prone to disruption by external factors, including antibiotic exposure. Studies have revealed both transient and persistent alterations to the adult gut microbiota community resulting from antibiotic treatment later in life. As antibiotics are routinely prescribed at a greater rate in the first years of life, the impact of these interventions on the developing IGM is emerging as a key research priority. In addition to understanding the impact of these disruptions on the infant gut microbial architecture and related host diseases, we need to understand the contribution of early life antibiotics to the selection of antibiotic resistance gene reservoirs in the microbiota, and their threat to successful treatment of infectious disease. Here we review the current understanding of the developmental progression of the IGM and the impact of antibiotic therapies on its composition and encoded reservoir of antibiotic resistance genes.

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Introduction

Antibiotics are the most prescribed medications in neonatal and pediatric populations in the United States [1–3]. In neonatal intensive care units (NICUs), ampicillin and gentamicin are prescribed twice as frequently as the next most common medication [2]. In children age 0–18, antibiotics are prescribed to more than 50% of individuals [1] and account for approximately 25% of prescriptions, with amoxicillin, azithromycin, and amoxicillin/clavulanate being the most common [3]. Antibiotic perturbation of the actively developing infant gut microbiota (IGM) has profound impacts on human health and disease throughout life, as alteration of the gut microbiota during this timeframe may disrupt metabolic and immune development [4••]. Equally important is the potential enrichment of the reservoir of antibiotic resistance genes ('resistome') available for transfer to pathogens [5], compromising treatment of infections in vulnerable populations. The phylogenetic and resistome composition of the IGM is connected, yet dynamic, with gut environment and antibiotic pressure increasing opportunities for horizontal gene transfer [6–8]. Until recently, the response of the IGM and its resistome to antibiotic perturbation was largely characterized by culture-based or PCR-based experiments [9•,10–12], which underestimate novel resistance genes. This response can be influenced by many factors, including antibiotic spectrum, duration, and delivery route (oral versus intravenous), as well as microbial community composition and antibiotic susceptibility. While it is clear that antibiotics disrupt the developing gut microbiota, eliminating taxa and enriching for antibiotic resistance genes (ARGs), we are just beginning to understand the relative contribution of each of these factors to the community-wide taxonomic and functional response to antibiotics.

Definitions and key concepts

Developmental progression: the normal patterned succession of bacterial species colonizing the infant gut in the absence of disruptive perturbation.
Antibiotic resistome: the collection of ARGs encoded in a microbial community.
Metagenomic functional selections: shotgun cloning and heterologous expression of microbial community DNA in model organisms to interrogate specific functions, for example, antibiotic resistance.
Preterm infant: infants born <33 weeks gestational age.
Very low birth weight infant: infants weighing <1500 g at birth.

Normal IGM and resistome development

The normal developmental progression of the IGM is patterned, yet highly dynamic and individual specific, and is shaped by many factors, including host physiology, genetics, diet, and environment [13,14••,15]. Upon birth, infants are exposed to a surge of microbes that colonize the epithelial surfaces, including the gastrointestinal system. The source and composition of this inoculating bacterial community is highly dependent on gestational age at time of delivery and, for term infants, mode of delivery [14••,16,17]. Term infants born vaginally are initially colonized by microbial communities resembling maternal vaginal microbiota (enriched in *Lactobacillus* and *Prevotella* spp.), while those delivered by caesarean section harbor communities that more closely resemble the skin microbiota (enriched in *Staphylococcus* and *Propionibacterium* spp.) [16]. For preterm infants (gestational age <33 weeks) the early gut microbiota composition resembles bacterial communities colonizing hospital surfaces and feeding and intubation tubing and are enriched in *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and *Escherichia coli* [18•]. Mode of delivery in preterm infants does not appear to significantly affect the initial colonizing community and is instead hypothesized to be highly influenced by environment [18•,19]. Following initial colonization, term and preterm IGM alike begin to increase in diversity with continual dynamic turnover in bacterial composition driven primarily by chronological age; however, specific bacterial succession patterns are unique to these two populations [13,14••,19]. The most notable difference in succession patterns between infant populations includes an enrichment in Proteobacteria at <2 weeks in preterm infants. A detailed time series of a single term infant revealed the developing IGM is initially dominated by Firmicutes, with low levels of Proteobacterial species introduced in the first week of life and persisting as minor components (<10% relative abundance on average) throughout the first 2.5 years of life [20•]. By contrast, preterm IGM are quickly dominated by Proteobacterial species within the first week of life and maintain high levels, comprising on average >75% relative abundance of the community, throughout the first month [14••,21]. In healthy term infants there is a dramatic increase in *Bifidobacterium* and *Bacteroides* spp. within the first six months of life. By the end of the first year of life the IGM begins to resemble an adult-like microbiota, reaching full maturity by 2–3 years of age [13,15,20•]. It is still unclear if preterm infants eventually follow a similar developmental pattern once 'caught up' to term infants in postmenstrual age (gestational age plus chronological age) or if this population is set on a unique developmental trajectory.

The functional capacity encoded in the IGM also changes dramatically in the first year of life. In term infants, a shift is observed from lactose metabolism when diet is comprised of human milk and formula, to polysaccharide utilization upon the introduction of solid foods [20•]. While the gut-associated resistome comprises epidemiologically important functions, less is known about how this reservoir of genes develops in early life. Recent studies have shown that ARGs in the IGM are established within the first week of life, even in the absence of antibiotic exposure [22,23••,24,57]. Most investigations of the early resistome have employed culture-based or PCR-based methods [9•,10–12]. Focusing on readily culturable bacteria and previously identified ARGs vastly underestimates the diversity and abundance of ARGs in the gut microbiome [5]. To overcome these challenges, a recent study used culture-independent methods to characterize the gut resistome of 22 healthy infants and children aged one month to 19 years [23••]. Employing high-throughput functional metagenomic selections [25], the authors demonstrate that the healthy pediatric gut resistome is established early in life and persists throughout childhood. Of the 18 antibiotics investigated, only gentamicin demonstrated age-discrimination independent of antibiotic exposure with children >12 months of age harboring significantly higher levels of gentamicin resistance compared to younger children [23••].

Early-life antibiotics and the human microbiota

Preterm or very low birth weight infants are at highest risk for antibiotic associated perturbations, as they routinely receive empiric antibiotic therapy at birth [26,27•]. As with adults, short-term perturbations of the IGM follow soon after antibiotic treatment, the broad characteristics of which are known through culture-based methods [28]. Recently, culture-independent methods for interrogating microbial communities have emerged, relying on DNA amplification and sequencing. When applied to the developing IGM, some studies suggest both phylogenetic diversity and microbial load are depressed following antibiotic therapy. For example, 16S rRNA-based phylogenetic profiling of fecal microbiota from preterm infants receiving ampicillin and gentamicin during the first week of life had lower diversity compared to un-treated infants [27•]. However, another study comparing the fecal microbiota composition of infants treated with oral cephalexin to infants receiving no treatment did not reveal significant differences during the month following therapy [29]. These differing findings may be due to different antibiotic regimens, routes of antibiotic administration, choice of statistical analytical methods, or other uncontrolled factors. The difficulties inherent to untangling these variables informs both the need for large cohort studies of specific antibiotic regimens and studies in controlled animal models. Bacterial load is another measure found to decrease in some studies but not in others. Quantitative PCR of 16S rRNA has been used to estimate bacterial load in the gut. In unrelated studies examining the IGM following antibiotic therapy, bacterial load was found to be unaffected, slightly altered, profoundly decreased, or