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Review

The resistance within: Antibiotic disruption of the gut microbiome and resistome dynamics in infancy

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SUMMARY

Intestinal host-microbiota interactions during the first year of life are critical for infant development. Early-life antibiotic exposures disrupt stereotypical gut microbiota maturation and adversely affect childhood health. Furthermore, antibiotics increase the abundance of resistant bacteria and enrich the resistome—the compendium of antibiotic resistance genes—within the gut microbiota. Here, we discuss acute and persistent impacts of antibiotic exposure during infancy on pediatric health, the gut microbiome, and, particularly, the resistome. Reviewing our current understanding of antibiotic resistance acquisition and dissemination within and between microbiomes, we highlight open questions, which are imperative to resolve in the face of rising bacterial resistance.

INTRODUCTION

During the first weeks of life, neonates are colonized with a diverse set of bacteria, viruses, fungi, and archaea (Gasparrini et al., 2019; Gibson et al., 2016; Rao et al., 2021; Yassour et al., 2016). The collection of these microbes and their functions—the microbiome—diversify and mature under environmental selection (Gasparrini et al., 2019; Yassour et al., 2016). During early life, critical host-microbe interactions at the intestinal interface promote healthy immune and metabolic development.

In high-income countries, eighty percent of children receive antibiotics during the first 48 months of life (Aris et al., 2021; Leong et al., 2020). In low-income and middle-income countries, the prescription rate is even higher, and children are estimated to receive an average of 11 antibiotic courses in the first 2 years of life (Fink et al., 2020). In high-income countries, the majority of exposures occur in the first year of life (infancy), during which 40%–70% of all infants receive at least one course of antibiotics (Dawson-Hahn and Rhee, 2019; Slykerman et al., 2017). Although antibiotics are among the most important medical advances in the last 100 years and have substantially decreased global mortality due to infectious disease, their overuse has accelerated the spread of resistance among bacterial populations. Moreover, they can also disrupt microbiome structure and function, often resulting in dysbiotic microbiome states associated with adverse health outcomes (Tamburini et al., 2016).

Our growing understanding of the gut microbiome has revealed the collateral damage of antibiotic use on the complex ecosystem of bacteria colonizing the gastrointestinal tract. Compositional and functional changes of the microbiome following antibiotic exposure result from differences in bacterial drug sensitivity determined either by the expression of antibiotic resistance genes (ARGs) or antibiotic concentration-dependent effects in the gut (Gibson et al., 2016; Maier et al., 2021). The collection of ARGs—the resistome—is a core characteristic of the microbiome. Pathogens can acquire ARGs from less pathogenic members of the microbiota via horizontal gene transfer (HGT), potentially gaining antibiotic resistance phenotypes of immediate clinical impact. While environment, maternal health, diet, birth mode, and gestational age can all affect maturation of the gut microbiome in infancy (Robertson et al., 2019), here we focus on antibiotic exposure and its impact on the gut microbiome and resistome during early life. Antibiotic exposure is a major determinant of the diversity and composition of the infant gut resistome (Gasparrini et al., 2019; Gibson et al., 2016). The effect of antibiotics is impacted both by host-intrinsic and extrinsic factors including, but not limited to, age, co-morbidities, genetics, and microbiome composition, as well as diet and environment, and the spectrum, route, duration, and history of exposure to the antibiotic (Gibson et al., 2016; Maier et al., 2021; Rooney et al., 2020; Tamburini et al., 2016).

While our knowledge about the dynamics that shape resistome establishment and the effects of antibiotics on the microbiome in early life has improved in recent years, critical questions about the inter-bacterial dynamics that contribute to the spread of resistance within bacterial communities, the genome-resolved functional impact of antibiotics in early life, and the clinical consequences of resistance within the microbiome remain unanswered. In this review, we summarize knowledge of the impact

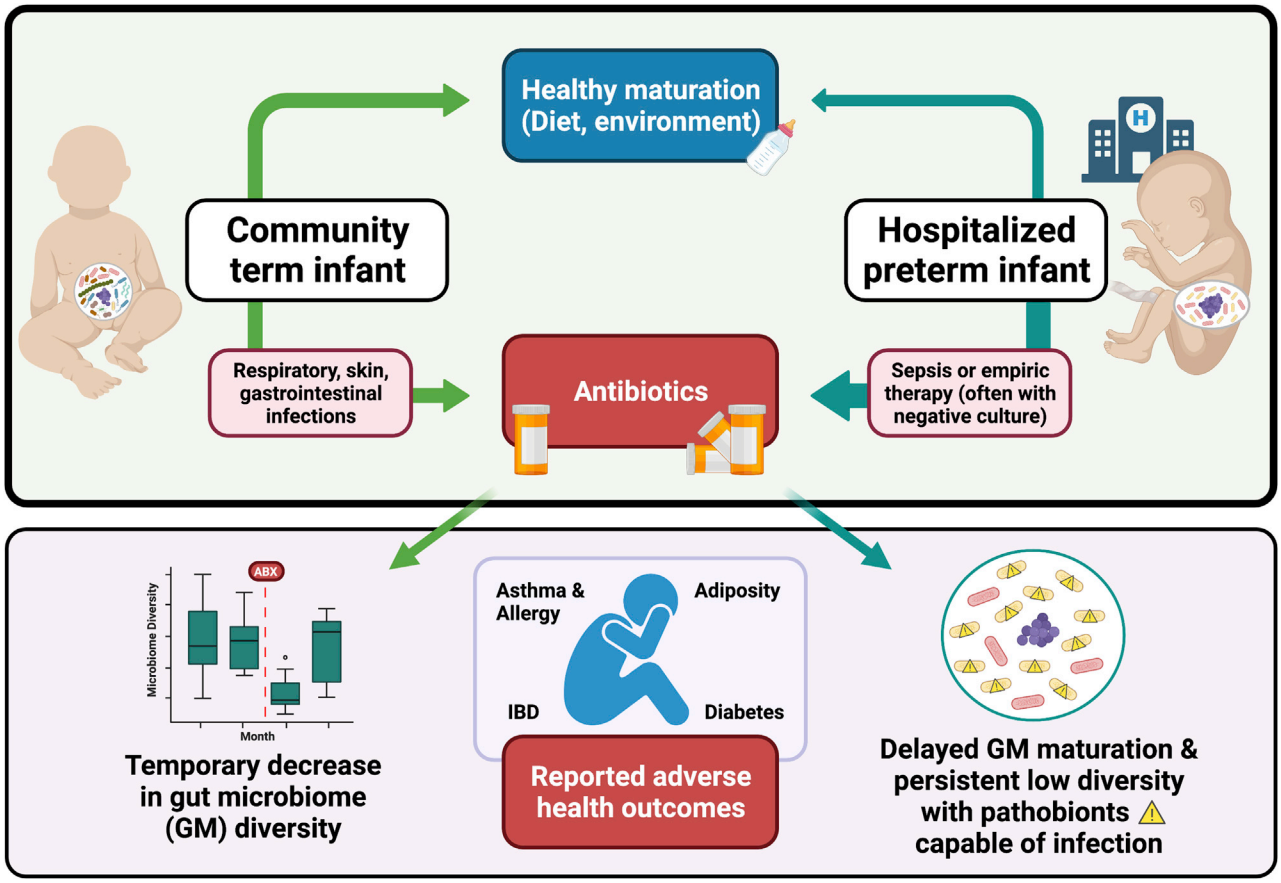

Figure 1. Indications and acute and long-term effects of early-life antibiotic exposure on gut microbiome trajectory and health outcomes in term and preterm neonates

40–70% of all term infants in high-income countries receive an antibiotic course in the first year of life, typically following community-acquired infection. Exposure results in a temporary decrease in gut microbiome diversity compared with antibiotic-naive infants. Most preterm infants receive empiric antibiotic therapy in the NICU, resulting in persistent low diversity microbiomes in the first year of life and sustained colonization with multidrug resistant pathobionts capable of causing infection. For all infants, antibiotic exposure in infancy is associated with adverse childhood health outcomes. IBD: inflammatory bowel disease. Created with BioRender.com.

of antibiotics on childhood health outcomes, the infant microbiome and resistome, and outline future avenues of research.

ANTIBIOTIC EXPOSURE AND HEALTH OUTCOMES IN EARLY CHILDHOOD

In early childhood, respiratory, skin, and gastrointestinal infections are the most common indications for use of antibiotics (Figure 1), but retrospectively, in 20%–40% of cases prescriptions are found to be inappropriate according to medical guidelines corresponding to a patient's diagnosis (Chua et al., 2019; Fleming-Dutra et al., 2016; Uda et al., 2019). While most pediatric antibiotic prescriptions occur in the outpatient setting (Gerber et al., 2021), exposure is frequent and extensive in neonatal intensive care units (NICUs), where over 80% of preterm infants receive antibiotics within 72 h of birth (Flannery et al., 2018). Although the most common antibiotics administered in NICUs are narrow spectrum agents, such as ampicillin, gentamicin, and vancomycin, up to 26% of antibiotic-exposed neonates receive broader spectrum 3rd and 4th generation cephalosporins and carbapenems (Prusakov et al., 2021). Empiric antibiotic therapy for suspected infections is the main indication in these cases (Cantey et al., 2015; Flannery et al., 2018). In the majority of cases, negative bacterial culture results suggest alternate etiologies, but neonates often continue to receive antibiotics, contributing to their overuse (Cantey et al., 2015). Thus, antibiotic use is common in infancy regardless of the environment (Figure 1).

Antibiotic use has declined in NICUs over the last 20 years (Schulman et al., 2018). Where implemented, antibiotic stewardship programs have contributed to a more rapid decline in antibiotic prescriptions in the NICU compared with institutions without such programs (Schulman et al., 2018). More broadly, these programs have led to declining antibiotic prescriptions in both inpatient and outpatient pediatric populations in the United States over the last 10 years, continuing a trend that began in the 2000s (King et al., 2020; Vaz et al., 2014). While these are hopeful signs that antibiotic overuse can be curtailed (Gerber et al., 2021), recent epidemiological studies have found sustained high prescription rates in outpatient settings, rural communities,

low- and middle-income countries, and in NICUs worldwide (Abuali et al., 2019; Araujo da Silva et al., 2019; Prusakov et al., 2021).

Early-life antibiotic exposure has been associated with a multitude of long-term adverse health outcomes, including childhood asthma, obesity, inflammatory bowel disease, and impaired growth (Figure 1; Tamburini et al., 2016). Recent research, however, has called some of these associations into question. The lack of association between early-life antibiotics and autism or hyperactivity disorders and conflicting results for asthma in familial analyses stand in contrast to significant associations in case-control designs and implicate common household co-exposures as a potential confounder of previous analyses (Örtqvist et al., 2014; Slob et al., 2020, 2021). Nevertheless, as studies using genetically identical and environmentally controlled animal models provide mechanistic support for a direct link between early-life antibiotics and adverse health outcomes (Livanos et al., 2016; Lynn et al., 2018, 2021; Schulfer et al., 2019), further epidemiological research is required to resolve apparent discrepancies in human populations. Though the mechanisms underlying the impacts of antibiotics on many chronic health sequelae remain elusive, their impacts on the microbiome and host immune system are likely contributors.

EFFECTS OF ANTIBIOTICS ON THE GUT MICROBIOME OF HEALTHY INFANTS

At birth, all neonates have a low diversity gut microbiome (Gasparrini et al., 2019; Yatsunenko et al., 2012). In the first weeks of life, infants accrue microbial species, including key anaerobic symbionts from the *Bifidobacteriaceae*, *Clostridiaceae*, and *Lachnospiraceae* families, following individualized trajectories (Gasparrini et al., 2019; Vatanen et al., 2018). The early-life gut microbiome undergoes distinct developmental phases, during which weaning is an inflection point that accelerates microbiome maturation (Bäckhed et al., 2015; Roswall et al., 2021). Multiple factors reviewed elsewhere (Derrien et al., 2019), including delivery mode, sex, gestational age, antibiotics, diet, and environment shape the composition and trajectory of individual infant microbiomes. Antibiotics can stunt early life gut microbiome maturation, resulting in transiently delayed dynamics and potential regression relative to age-matched controls (Beller et al., 2021; Bokulich et al., 2016; Vatanen et al., 2018).

Emerging evidence implicates the first year of life as a critical window during which cues from the intestinal microbiota educate the developing infant immune system (Depner et al., 2020; Henrick et al., 2021; Olin et al., 2018). Infants with gut microbial dysbiosis in the first 40 days of life exhibited altered populations of circulating immune cells and increased T cell activity at 12 weeks (Olin et al., 2018). Recent work has further shown that individual microbial taxa, specifically Bifidobacteria, and their human milk oligosaccharide utilization gene cluster are key to educating intestinal T helper cell populations and preventing inflammation during the first months of life (Henrick et al., 2021). Microbiota disruption during this time can hamper immune development and is associated with adverse childhood health outcomes (Depner et al., 2020; Olin et al., 2018). Critically, the impact of antibiotic exposure is largest in the first year of life (Vatanen et al., 2018), resulting in attenuated gut microbiome maturation and diversity (Yassour et al., 2016). While these alterations are generally transient, some may persist long term (Gasparrini et al., 2019; Guittar et al., 2019), and the immunological importance of early life exacerbates the impact of even temporary perturbations (Olin et al., 2018).

Depletion of vulnerable taxa essential for host immune education may be at the core of observed lasting health impacts of early-life microbiota disruption (Henrick et al., 2021; Olin et al., 2018). Bifidobacteria and other anaerobic commensals known to modulate host-microbiota interactions in infancy are particularly vulnerable to antibiotic-induced replacement (Rooney et al., 2020; Vatanen et al., 2018). Data from animal models and observational studies suggest that transient early-life antibiotic-induced microbiota disruptions may indeed be at the root of long-term health consequences (Depner et al., 2020; Livanos et al., 2016; Lynn et al., 2018, 2021). Further studies at the interface of mucosal immunology and the gut microbiome are critical to identify elusive mechanistic links between transient gut microbiome disruptions and long-term health.

Recently, strain-resolved metagenomic analysis has suggested that community-level characterization may not reveal the full impact of antibiotics on the developing infant gut microbiome. Healthy, term neonates often accrue multiple strains of the same species that can differ in encoded functional potential. In contrast, the antibiotic-exposed infant gut microbiota exhibit less strain diversity compared with antibiotic-naive infants (Yassour et al., 2016). For example, *Bacteroides spp.* in antibiotic-exposed infants were often dominated by a single strain, unlike antibiotic-naive infants who harbor multiple strains of the same species, reflecting reduced within-species genomic variation and potentially significant functional effects following antibiotic exposure (Yassour et al., 2016). This effect is strongest for species seeded in a single colonization event (Yassour et al., 2016). Antibiotics can cause rapid shifts in the genomic composition of individual species that often occur without obvious changes in relative abundance, highlighting that these antibiotic-induced effects are missed by species-resolved analysis (Roodgar et al., 2021). It remains to be investigated whether antibiotic-induced strain depletion persistently impacts within-species genomic diversity, how taxonomic shifts impact the functional repertoire of keystone species, and whether strain shifts impact health outcomes.

EFFECTS OF ANTIBIOTICS ON THE GUT MICROBIOME DURING POSTNATAL HOSPITALIZATION

Infants born prematurely frequently require hospitalization in NICUs where gut microbiome diversity remains low for months and often comprises only a few species (Gasparrini et al., 2019; Gibson et al., 2016; Rao et al., 2021). Antibiotic-resistant *E. coli*, *Klebsiella spp.*, *Staphylococcus spp.*, and *Enterococcus spp.*, which are common etiologic agents of infant bloodstream infections (BSIs) (Carl et al., 2014), dominate the neonatal preterm gut microbiome and hospital environment in the first months of life (Brooks et al., 2017; Gibson et al., 2016; Rao et al., 2021). The preterm gut microbiome clusters into distinct community states dominated by one of these genera (Rao et al., 2021). Staphylococci are the dominant member in the first 7 days of life with high relative and absolute abundance (Rao

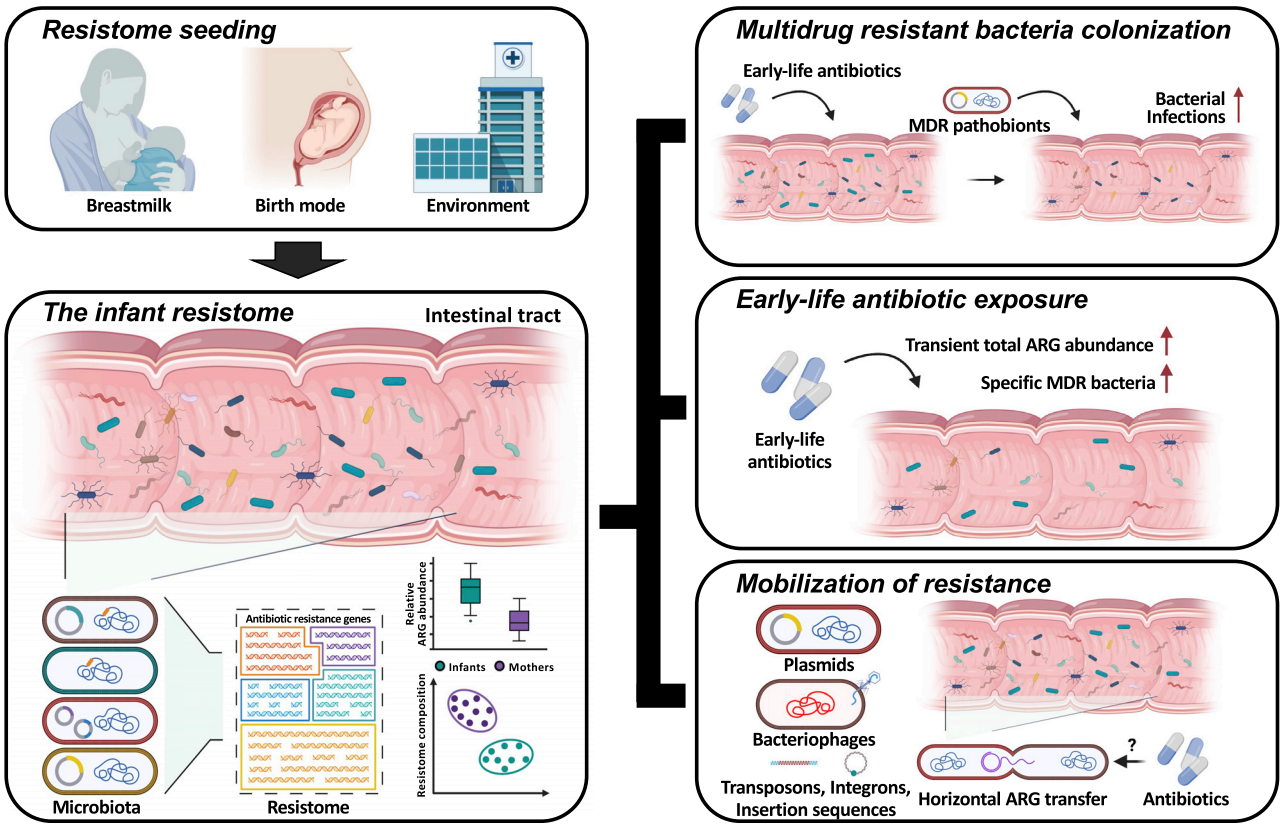

Figure 2. Resistome dynamics in infancy

The infant gut resistome, the collection of antibiotic resistance genes (ARGs) encoded by the gut microbiota, is seeded from maternal and environmental sources. Resistome burden is elevated in early life and compositionally different to infant mothers. Early-life antibiotics open niches for the colonization by pathobionts that are often multi-drug resistant (MDR) and associated with childhood infections, as well as increase resistome burden and abundance of gut endemic MDR bacteria. Furthermore, antibiotic resistance is mobilized on plasmids, bacteriophages, or via transposons, integrons, and insertion sequences. These contribute to the spread of ARGs within gut communities, potentially triggered by antibiotic exposure. Created with BioRender.com.

et al., 2021). This initial stage can be followed by an influx of *Klebsiella spp.* exploiting physiological niches and repressing the abundance of Staphylococci. Alternatively, if Enterococci are present, they repress *Klebsiella spp.* abundance (Rao et al., 2021). Thus, the dynamics of hospitalized preterm infant gut microbiomes are characterized by opportunistic, often drug-resistant pathobionts, differing significantly from the dynamics observed in their non-hospitalized term counterparts (Gasparrini et al., 2019).

In premature infants, increased antibiotic duration, spectrum, and frequency correlate with decreased overall diversity and increased abundance of potentially antibiotic-resistant gram-negative organisms, such as *E. coli*, *Enterobacter spp.*, and *Klebsiella spp.* (Gasparrini et al., 2019; Gibson et al., 2016; Thänert et al., 2021). The impact of antibiotics on preterm gut microbiome diversity and richness is, however, not uniform, as most antibiotic classes exhibit specific inhibition spectra (Maier et al., 2021). Generally, broader spectrum antibiotics, such as carbapenems and 3rd generation cephalosporins, decrease intestinal species richness more than narrower agents, such as ampicillin or vancomycin (Gibson et al., 2016). However, the impact of each antibiotic on each neonate is not uniform, leading to variability in post-antibiotic microbiome compositions (Gibson et al., 2016). Many antibiotics have anti-anaerobic activity (Maier et al., 2021), preventing anaerobes critical for infant immune education from gaining a foothold in the preterm gut (Rooney et al., 2020). Each additional day of ampicillin, cefotaxime, tobramycin, and/or metronidazole exposure in the NICU is associated with a 16% decrease in anaerobic species (Rooney et al., 2020). Taken together, the specific antibiotic, duration, and patient gut microbiota composition interact, resulting in individualized responses to antibiotic interventions in the NICU. While the acute impact of antibiotics in the NICU have been studied, there remains, with few exceptions (Gasparrini et al., 2019), a paucity of long-term follow up studies investigating health outcomes and lasting microbiota effects to early-life antibiotic exposures. Such studies would inform us of risk-benefit assessment of empiric therapies by pediatricians and potentially impact overall health outcomes in this vulnerable population.

THE RESISTOME IN EARLY LIFE

Early life reflects a period of elevated resistome burden (Figure 2), demonstrated by greater relative ARG abundance in the gut microbiomes of infants relative to those of their adult mothers (Pärnänen et al., 2018; Yassour et al., 2018). At this age, efflux

pumps often conferring multi-drug resistance (MDR) comprise the majority of the resistome and—along with aminoglycosides, bacitracin, beta-lactams, colistin, quinolones, and sulphonamide resistance classes—are found at higher relative abundance compared with their mothers (Li et al., 2021; Pärnänen et al., 2018; Rahman et al., 2018). The elevated ARG burden is most pronounced in the first weeks after birth and abates throughout the first year of life, primarily through decreasing abundance of efflux pumps (Lebeaux et al., 2021).

The expanded early-life resistome is linked to the taxonomic composition of the gut microbiota (Gasparrini et al., 2019; Gibson et al., 2016; Lebeaux et al., 2021; Li et al., 2021; Pärnänen et al., 2018; Yassour et al., 2016). Thus, the relative abundance of specific ARGs often rises rapidly during antibiotic treatment and falls immediately upon cessation, and such spikes are closely correlated with commensurate changes in the abundance of specific taxa (Gibson et al., 2016; Yassour et al., 2016). *Escherichia coli* is thought to be a main contributor to the increased ARG burden in infancy, as its abundance strongly correlates with that of an infant's resistome (Gasparrini et al., 2019; Lebeaux et al., 2021; Li et al., 2021; Pärnänen et al., 2018). Indeed, a recent study has found that *E. coli* is the putative bacterial host of 36 of the 50 most abundant ARGs in the infant microbiome (Lebeaux et al., 2021). When infant gut microbiomes are categorized by either low or high ARG richness and diversity, *E. coli* abundance is the main variable distinguishing the two (Li et al., 2021). 94% of the total abundance of the 58 most variable ARGs between the two groups were found on *E. coli* metagenome-assembled genomes (MAGs) (Li et al., 2021), substantiating the disproportionate impact of *E. coli* on the infant resistome. Notably, a recent meta-analysis has demonstrated that microbiomes dominated by Staphylococci can have similarly high relative ARG abundances (Pärnänen et al., 2022), highlighting that *E. coli* is not the sole correlate of elevated resistome burdens in early life. Moreover, it is important to note that the relative lack of genomic investigation of hard-to-culture gut microbiota may cause their contribution to the early-life resistome to be underestimated.

Determinants of infant gut resistome seeding and development are understudied (Figure 2). Evidence for intergenerational transfer of antibiotic-resistant strains, which was reviewed recently (Patangia et al., 2022), remains scarce. There is observational evidence that antibiotic-resistant bacteria colonizing the maternal vagina, including extended-spectrum beta-lactamase producing and MDR *E. coli*, can be transferred to neonates during vaginal birth (Patangia et al., 2022). In the first weeks of life, cesarean birth is associated with an increased resistome burden, specifically enriching glycopeptide, phenicol, pleuromutilin, bacitracin, sulfonamide, and diaminopyrimidine ARG abundance (Busi et al., 2021; Lebeaux et al., 2021). However, this association subsides in the first year of life (Busi et al., 2021; Li et al., 2021; Sosa-Moreno et al., 2020), indicating that the impact of cesarean birth may be related to the altered dynamics of intergenerational microbiota transfer and the prolonged exposure to the hospital environment compared with vaginal-born neonates. Intrapartum antibiotic therapy, often suggested as another effector of resistome development, has not been shown to impact overall resistome abundance or composition (Lebeaux et al., 2021; Pärnänen et al., 2018).

In early life, infants are fed a diet of breastmilk, formula, or a combination of the two. During this time, formula feeding has been associated with a significantly greater relative resistome burden (Pärnänen et al., 2022). Breastmilk has been found to contain antibiotic-resistant Bifidobacteria, Lactobacilli, Staphylococci, and Enterococci that can be transferred to infants (Patangia et al., 2022). In fact, exposure to breastmilk has a sustained effect on the resistome composition throughout infancy (Rahman et al., 2018; Sosa-Moreno et al., 2020). Consequently, infant gut resistomes share ARGs with maternal gut and breastmilk resistomes (Pärnänen et al., 2018). However, studies differ in their assessment of the magnitude to which the maternal microbiome seeds the infant resistome (Pärnänen et al., 2018; Sosa-Moreno et al., 2020; Yassour et al., 2018), potentially due to contrasting methodological approaches. Future genome resolved-studies at the strain level that link ARGs to their bacterial host—for example using Hi-C technology (Kent et al., 2020)—can resolve this open question.

Early-life antibiotic exposure is not necessary for resistome development. Even antibiotic-naive infants harbor ARGs and MDR bacterial strains in their gut microbiome in the first months of life (Gasparrini et al., 2019; Pärnänen et al., 2018; Yassour et al., 2016). This may be a consequence of transgenerational transmission, colonization from environmental sources, or a survival-based response of the gut microbiota to antimicrobials produced by normal gut inhabitants (Pärnänen et al., 2018; Yassour et al., 2016). Notably, ARGs conferring resistance to antibiotics that are not commonly used in neonatal populations are found in most infant gut microbiomes. Functional metagenomics identified ARGs against chloramphenicol and tetracyclines in a cohort of antibiotic-exposed preterm infants that were never exposed to these drugs during their NICU stay (Gibson et al., 2016). A follow-up study also found chloramphenicol ARGs in the gut microbiome of fully antibiotic-naive infants during the first 2 years of life, as well as resistance against "last line of defense" drugs colistin and tigecycline in both antibiotic-exposed and -naive infants (Gasparrini et al., 2019). These observations have been confirmed inindependent work (Li et al., 2021; Pärnänen et al., 2018), highlighting that the resistome is a core characteristic of the infant gut microbiome rather than a feature that arises solely in response to antibiotic selection.

Nevertheless, ARG richness and resistome burden correlate with cumulative antibiotic exposure in the first weeks of life (Gasparrini et al., 2019). Preterm infants who receive multiple courses of antibiotics during their NICU stay have a significantly higher resistome burden and richness relative to age-matched antibiotic-naive near-term infants (Gasparrini et al., 2019; Gibson et al., 2016). Furthermore, their resistome composition is distinct from that of antibiotic-naive infants (Gasparrini et al., 2019), mostly driven by changes in abundance of specific taxa in response to antibiotic exposure. Studies have reported *Klebsiella*-driven surges of beta-lactamase genes during penicillin exposure (Yassour et al., 2016), as well as significant increases in ARGs as a consequence of *S. epidermidis* and *K. pneumoniae* blooms following meropenem and ticarcillin-clavulanate exposure, respectively (Gibson et al., 2016), indicating that hospitalization and early-life antibiotics directly alter resistome composition. Importantly, if not repeatedly prescribed, the effect of antibiotics on the pediatric resistome is likely to be transient, as the impact of even broad spectrum agents given in the first week after birth subsides by 1 year of life (Reyman et al., 2022).

MULTIDRUG RESISTANT ORGANISMS IN THE INFANT GUT

Early-life antibiotic exposure has been linked to persistent gut colonization by MDR organisms (Figure 2), defined as bacteria resistant to more than three antibiotic classes (Gasparrini et al., 2019; Thänert et al., 2021). As highlighted above, these may be seeded intergenerationally or acquired environmentally (Brooks et al., 2017; Patangia et al., 2022). In preterm infants, prolonged hospitalization has been linked to the acquisition of Enterococci and *Enterobacteriaceae* strains from the hospital environment (Brooks et al., 2017), many of which are MDR and can persist in the gut following hospital discharge (Gasparrini et al., 2019). Rising antibiotic resistance among neonatal pathogens may thus further exacerbate the long-term health impact of early life hospitalization (Flannery et al., 2021). Notably, persistence of MDR bacteria throughout infancy was similarly observed in at least one antibiotic-naive term infant (Gasparrini et al., 2019), suggesting that hospitalization and early-life antibiotics may increase the likelihood of the initial acquisition but are not a prerequisite for MDR strain persistence.

The presence of MDR pathobionts in the gut microbiome is associated with adverse health outcomes. Intestinal colonization with Enterococci and *Enterobacteriaceae* in the NICU and after discharge is associated with bacterial BSIs (Carl et al., 2014; Thänert et al., 2021), and similar strains of *Enterobacteriaceae* inhabit the gut prior to BSI (Carl et al., 2014). In pediatric short bowel syndrome, strains of BSI-causing Enterococci and *Enterobacteriaceae* can persist in the intestine late into childhood (Thänert et al., 2021). During years of colonization, these strains can cause recurrent BSIs while persisting as minor members of the intestinal flora (Thänert et al., 2021). While the unique intestinal characteristics and repeated antibiotic exposures accompanying short bowel syndrome likely facilitate pathobiont persistence, such findings illustrate the risk associated with intestinal pathobiont persistence in pediatric populations. However, while it can be assumed that MDR in intestinal pathobionts can have significant impacts on treatment outcomes, studies investigating the specific impact of MDR pathobionts acquired in early life on childhood health remain elusive. Therefore, comprehensive identification of factors governing early-life acquisition and persistence of MDR organisms are necessary to decrease the risk of serious adverse health outcomes in infancy and throughout childhood.

MOBILIZATION OF RESISTANCE IN THE INFANT GUT

Mobile genetic elements (MGEs)—including plasmids, transposons, integrons, and insertion sequences—are responsible for the spread of ARGs intracellularly and between bacterial cells in the gut microbiome (Figure 2; Ebmeyer et al., 2021) and may underlie the sustained increase in abundance of certain ARGs following early-life antibiotic exposure (Yassour et al., 2016). Conflicting reports exist as to the extent of maternal contribution to infant mobilome seeding. Mobilome composition is more similar between unrelated infants than mother-infant dyads, and MGE abundance and diversity in infants is increased relative to their mothers despite significantly reduced taxonomic diversity (Pärnänen et al., 2018; Sosa-Moreno et al., 2020), implying taxa that are overrepresented in the infant gut disproportionately harbor MGEs. Indeed, *Enterobacteriaceae*, the predominating family and oversized contributor to resistome burden in the first months of life (Gibson et al., 2016), are also frequent MGEs carriers, illustrating the intimate connection between the resistome and the mobilized genetic content, or "mobilome," and shared bacterial hosts (Gasparrini et al., 2019; Gibson et al., 2016; Lebeaux et al., 2021; Li et al., 2021; Pärnänen et al., 2018).

Spatial association between MGEs and ARGs in the infant gut is infrequent but can have broad clinical consequences. Functional metagenomic selection of infant stool metagenomes against 16 clinically relevant antibiotics found that 6.4% of ARGs were syntenic or located in proximity to an MGE, commonly a transposase or integrase (Gasparrini et al., 2019). This observation is supported by reference-based and predictive computational analysis of 20 infant gut metagenomes that found that 6%–8% of ARGs are located on plasmids or bacteriophages (Busi et al., 2021). ARG-MGE associations allow for the mobilization of resistance that contributes to the rise of drug-resistant infections; however, to date there is a dearth of studies comprehensively describing the relationship between specific ARG and MGE classes within drug-resistant strains in the infant gut microbiome.

Though tetracyclines are rarely prescribed during infancy, tetracycline resistance genes are frequently transferred intergenerationally and are potentially mobilizable (Sosa-Moreno et al., 2020). Tetracycline resistance genes, including *tetX*, are the most commonly shared ARG class between related mother-infant dyads (Sosa-Moreno et al., 2020; Yassour et al., 2018) and co-localize with MGEs in the infant gut microbiome (Gasparrini et al., 2019; Pärnänen et al., 2018), highlighting their elevated potential for vertical and horizontal transfer. Inter-species transfer in the gut microbiome has been reported for multiple resistance classes (Busi et al., 2021) and may be triggered by antibiotic selection (Kent et al., 2020). Transfer of ARGs in human microbiomes may in fact follow a predictable framework governed by the presence of key functions, including toxin-antitoxin and restriction modification systems (Zhou et al., 2021). Recent research has shown frequent ARG transfer in the gut microbiome of adults (Kent et al., 2020), though similar analyses in pediatric cohorts have yet to be performed. Given its enrichment of ARGs, MGEs, and pathobionts, the infant gut microbiome is a potential hotspot for the inter-species spread of antibiotic resistance, highlighting the need for and clinical importance of further investigation.

FUTURE DIRECTIONS

In the face of surging resistance, even against antibiotics of last-resort in pathogenic bacteria inhabiting the infant gut (Gasparrini et al., 2019), there is an urgent need to further our understanding of the early-life resistome. Here, we highlight aspects of resistome research with immediate clinical importance in pediatric populations.

Seminal research leveraging the power of high-throughput metagenomic sequencing coupled to large human cohorts has significantly advanced our understanding of the factors that govern microbiome assembly in infancy. Comparably, less is known about the resistome in early life. While multiple factors

impact resistome seeding and composition in early life, including gestational age, birth mode, breastmilk, environment, and antibiotic exposures (Brooks et al., 2017; Gasparrini et al., 2019; Lebeaux et al., 2021; Li et al., 2021; Pärnänen et al., 2018; Patangia et al., 2022), less is known about how they affect seeding and persistence of high-risk MDR pathogens in the pediatric gut (Gasparrini et al., 2019). Recent work has found that *E. coli* is the largest contributor to the gut resistome in healthy infants (Lebeaux et al., 2021; Li et al., 2021) but lacked investigation of its initial sourcing or persistence of specific drug-resistant strains through infancy. Strain-resolved studies identifying sources as well as factors impacting persistence of high-risk MDR pathogens throughout childhood could enable development of novel interference strategies aimed at mitigating the risk associated with MDR pathogen gut colonization. Microbiota-humanized animal models may serve as a promising avenue to interrogate the impact of strain-resolved inter-bacterial interactions on resistome seeding and ARG spread in the gut microbiome.

The risk of individual ARGs for human health is closely tied to the pathogenic potential of its bacterial host, as well as its potential to facilitate resistance against antibiotics commonly used in empiric therapy. Therefore, we propose the need for comprehensive assessment of the risk associated with the presence and host-association of ARGs within the infant gut microbiome. Assigning risk scores based on frequency of observed treatment failures to host-ARG pairings could identify associations of high clinical relevance in diverse pediatric populations. Further, coupling this risk to predictions of ARG dissemination across bacterial species within patient-hospital transmission networks would allow the development of strategies limiting the clinical impact of high-risk ARGs. Recent work has highlighted CRISPR-Cas9-based targeted genome editing using diverse delivery systems as a potentially revolutionary approach to microbiome manipulation (Lam et al., 2021). This system can facilitate strain-specific depletions and genomic deletions *in vivo* and thus has great potential for the targeted depletion of both MDR strains as well as high-risk ARGs and MGEs from the infant microbiome. Similarly, dietary interventions or fecal microbiota transplants could be used to alleviate infants of high resistome burdens and persistent low diversity microbiome states.

Finally, recent technological advances have opened new horizons in the field of rapid bacterial diagnostics. A groundbreaking work used the MinION sequencing platform for analysis of fecal metagenomic DNA from preterm infants, identifying intestinal carriage of pathogens and their resistance profile in near real-time (Leggett et al., 2019). This technology has the potential to inform treatment decisions specifically in the context of hospitalized preterm infants at risk of hospital-acquisition of MDR pathogens (Brooks et al., 2017; Gibson et al., 2016). Together these studies and technological advances have the potential to revolutionize our understanding of the early life microbiome and resistome, as well as its clinical importance, and equip us with tools to protect infant health in the face of rising resistance.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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