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REVIEW

Early-life antibiotic usage and impact on the gut microbiota, including emergence of antimicrobial resistant *Enterococcus*

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Abstract

The early-life microbiota is an 'immature' and highly dynamic microbial ecosystem, which is central to infant health. Both perinatal and postnatal factors can impact the gut microbiota, with antibiotics proposed to cause short and longer-term disturbances. Antibiotics not only impact microbial community composition but also contribute to the overall antibiotic resistance profile, i.e. the 'resistome', and they may also enhance carriage of multi-drug-resistant bacteria. Given high antibiotic prescription practices in pregnant women and newborns this also contributes to the global threat of antimicrobial resistance. This review summarises the current literature on antibiotic usage and how this may impact the developing gut microbiota during early-life, including the influence of horizontal gene transfer on contributions to pathogenicity and resistance of gut bacteria. We also focus on *Enterococcus* spp. given their high levels in infants and their link with opportunistic infections that are a significant cause of morbidity and mortality during early-life. Finally, a perspective on the importance to antibiotic stewardship, and harnessing the microbiota itself for anti-infection therapies for reducing antibiotic usage are also covered.

Keywords

- early-life microbiota
- antimicrobial resistance
- gut microbiome
- resistome
- antibiotics
- horizontal gene transfer
- MGEs
- plasmids
- *Enterococcus*

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Antibiotic consumption and antimicrobial resistance – the current problem

Antimicrobial resistance (AMR) is an ongoing pandemic and its burden relies on over and misuse of antibiotic drugs. A recent systemic analysis indicated deaths associated with bacterial AMR in 2019 was 4.95 million, including 1.27 million deaths directly attributable to bacterial AMR (Murray *et al.* 2022). High antibiotic prescription rates raise the concern for AMR in high-income countries (HICs), and one in three people in WHO European Region (14 countries) used leftover antibiotics from a previous prescription or obtained antibiotics without a prescription (WHO 2023) (Torres *et al.* 2021, Ndaki *et al.* 2022). In low- to middle- income countries (LMICs) this is further compounded by easier access to antibiotics in community settings. A review on AMR by O'Neill forecasted more 10 million deaths due to AMR in 2050 with Asia and Africa most affected, followed by Europe, Latin America and North America. This report also highlights important factors like severe delay of commercialisation of new antibiotics in HICs, and

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poor stewardship programs in LMICs and in HICs (O'Neill 2014).

Crucially, overuse of antibiotics not only increases risk of AMR, but can also increase the severity and length of disease, which in turn increases healthcare costs and mortality rates due to emergence of multi-drug-resistant (MDR) bacteria (Llor & Bjerrum 2014). In paediatric populations, the WHO estimates AMR causes 200,000 infants' deaths every year (Romandini *et al.* 2021). About 40% of newborn infections are resistant to standard antibiotic treatment globally, suggesting a reservoir of MDR bacteria and antibiotic-resistant genes in this population (WHO 2023). Primary care accounts for more than 80% of all antibiotic prescriptions across Europe, which are mostly for respiratory infections (Llor & Bjerrum 2014). Recent studies also indicate that 33–39% of newborns are exposed to antibiotics via the mother (to prevent preterm birth and reduce the risk for maternal or neonatal infections) during delivery (Stokholm *et al.* 2013). In the last decade, antibiotic consumption has rapidly increased in LMICs (Sulis *et al.* 2020, Kwon *et al.* 2022), with inadequate testing facilities, hygiene and antibiotic storage conditions, potentially worsening the AMR situation (Ayukekbong *et al.* 2017). Table 1 shows the most commonly prescribed/used antibiotics in different global regions. Although educational interventions, particularly in HICs, have been associated with reductions in antibiotic consumption and promotion of appropriate antibiotic prescription practices, ongoing and further efforts are crucial in this area (Pierce *et al.* 2020, Pinto Ferreira *et al.* 2022, Rocha *et al.* 2022).

A multinational survey conducted across 76 countries reported antibiotic consumption rates between 2000 and 2015. The consumption of Access (essential antibiotics that are first or second line of treatment for common infections) and Watch (antibiotics that are only applied to limited group of infective syndromes) antibiotics increased by 26.2% and 90.0%, respectively. In LMICs, the consumption of Access antibiotics was 45.3%, with Watch antibiotics increasing by 165%, while in HICs it was 14.8% and 27.9%, respectively, across the same 15-year period. Countries in which Access antibiotics made up at least 60% of their antibiotic consumption decreased over the study period, from 50 out of 66 countries in 2000, to 42 out of 76 countries in 2015. These data indicate an antibiotic usage alarming situation, and the WHO Aware framework and nationwide target for Access antibiotics is now aimed at least 60% of overall antibiotic consumption by 2023 (Klein *et al.* 2021).

Notably, although over/misuse of antibiotics is associated with driving AMR – there is also significant challenges associated with inaccessibility to medicines, including antibiotics. Indeed poor access to antibiotics was associated with increased pneumonia and acute febrile illness among children less than 5 years of age (Sulis & Gandra 2021). In terms of access to new antibiotics, a study found that the majority of antibiotics approved and launched between 2010 and 2020 were accessible in only 3 (US, UK and Sweden) out of 14 HICs, with the situation significantly worse in LMICs. Indeed, only 10 out of 25 new antibiotics that entered the market between 2010 and 2019 were registered in more than 10 countries. With low sales, many companies have delayed commercialisation fearing decreases in profitability (Kållberg *et al.* 2018, Outterson *et al.* 2021). The main barriers to access of new antibiotics includes affordability, insufficient government funding, weak drug discovery, difficulties in market entry and vulnerable health systems (Frost *et al.* 2019). In order to combat these issues, and in the face of AMR, the Global Antibiotic Research and Development Partnership (GARDP) is developing treatments for (multi)drug-resistant infections, including working together with partners to develop new antibiotics,

Table 1 Top most prescribed antibiotics given to infants in different geographical regions. These antibiotics were prescribed for conditions such as ear infections, necrotising enterocolitis (NEC), urinary tract infection (UTI), respiratory tract infection, pneumonia, meningitis and sepsis.

Regions	Antibiotics	References
Europe	Ampicillin, gentamicin, linezolid, vancomycin	Prusakov *et al.* (2021), Nasso *et al.* (2022)
Asia	Carbapenems, tetracycline, gentamicin, amoxicillin	Hufnagel *et al.* (2018), Bielicki *et al.* (2020)
Australia	Gentamicin, benzylpenicillin (penicillin G), cefotaxime, ampicillin	Anderson *et al.* (2017), Yan *et al.* (2019), McMullan *et al.* (2020)
USA	Ampicillin, gentamicin, vancomycin, cefazolin	Poole *et al.* (2019), Spencer *et al.* (2022)
Africa	Cephalosporins, penicillin, carbapenems, gentamicin	Bielicki *et al.* (2020), Abubakar & Salman (2023), Mambula *et al.* (2023)

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and improving antibiotics access in a responsible way (Russell *et al.* 2023).

Impact of antibiotics on early-life gut microbiota composition

Gut-associated microbial communities i.e. the gut microbiota, are seeded at birth, and play a critical role in early-life development. Composition of the early-life microbiota can either be associated with health or disease, with colonisation by beneficial microbes at key stages required for host wellbeing (Milani *et al.* 2017).

Intrinsic and extrinsic factors play a significant role in shaping interactions between gut microbes, and also how these microbes impact host responses. These factors include host genetics, antibiotic use, maternal and infant nutrition, gestational age, and mode of delivery, all of which can impact gut microbiota signatures in the short and longer term (Fouhy *et al.* 2019). In general, vaginally born infants have dominant genera such as *Bifidobacterium, Bacteroides, Parabacteriodes* and *Escherichia*, which are transferred vertically from maternal vaginal and gut sites, while caesarean-born infants can harbour potentially pathogenic taxa including *Enterococcus, Staphylococcus, Streptococcus, Klebsiella, Clostridium,* and *Enterobacter* that may originate from the mother's skin and hospital environments (Shao *et al.* 2019, Chong *et al.* 2022). Breast milk contains human milk oligosaccharides (HMOs), which are not digested by host but metabolised by *Bifidobacterium* spp. that are enriched in breast-fed infants (Tamburini *et al.* 2016). Contrastingly, formula-fed infants have a more diverse microbiota – with higher prevalence of opportunistic pathogenic taxa in the gut (Ma *et al.* 2020, Laursen 2021).

Although birth mode and nutrition significantly alter the developing gut microbiota, antibiotics also have wide-ranging effects on taxonomic composition, which is further compounded by the issue of antibiotic over/misuse and AMR as highlighted above. Indeed, it is estimated that only 1 in 1000 neonates that receive antibiotics will develop an officially diagnosed infection, and currently antibiotics are prescribed in 4–10% of all neonates (Reyman *et al.* 2022).

Prior to birth, intrapartum antimicrobial prophylaxis (IAP) is given to women during onset of labour and delivery to reduce group B Streptococcal infections (Braye *et al.* 2019). IAP antibiotics includes intravenous penicillin or ampicillin, with cefazolin recommended for women with a penicillin allergy (Braye *et al.* 2019). IAP-exposed infants have been shown (at 3 months) to have low levels of *Bacteroides* and *Parabacteroides* and high levels of *Enterococcus* and *Clostridium* (Patangia *et al.* 2022).

After birth, a group of infants that are often 'overexposed' to antibiotics are preterm infants, who often receive broad-spectrum antibiotics during their hospital stay, with up to 80% exposed to antibiotics in their first postnatal week (Bubser *et al.* 2022). Commonly used antibiotics are gentamicin, penicillins and vancomycin (Simeoli *et al.* 2022). An estimated 15 million infants are prematurely born every year and 1 million children die each year due to complications, including infections (WHO 2023). Previous studies have indicated low to absent levels of *Bifidobacterium* and *Bacteroides,* with high levels of facultative anaerobes such as Enterobacteriaceae and increased levels of *Clostridium*, *Streptococcus* and *Staphylococcus* (Shao *et al.* 2019). Crucially, these perturbed gut microbiota profiles in infants is associated with the devastating gut disease NEC, which is associated with overgrowth of opportunistic pathogens (that are often MDR) in the preterm gut (Cuna *et al.* 2021). Specifically, microbial perturbations preceding NEC have been identified, with increases in the abundance of Enterobacteriaceae, *Enterococcus*, and *Citrobacter*, with concurrent decreases in *Bacteriodetes* and *Veillonella* levels (Pammi *et al.* 2017). A more recent shotgun metagenomic study of NEC patients revealed that *Enterococcus faecalis* and *Escherichia coli* were the dominant taxa, followed by *Staphylococcus epidermidis* and *Klebsiella pneumoniae* (Tarracchini *et al.* 2021). Most recently, a link between *Clostridium perfringens* and preterm infants with NEC has been postulated, which was confirmed by in-depth genomics and experimental characterisation (Kiu *et al.* 2023).

Unlike preterms, full-term infants tend to spend less days in hospital (Fuertes *et al.* 2023). However, they also receive antibiotics, with ampicillin, gentamicin and vancomycin the most commonly prescribed (Leroux *et al.* 2015, Rivera-Chaparro *et al.* 2017, Prusakov *et al.* 2021). Antibiotics during these first days and weeks shape the diversity and composition of the term infant microbiota (Shekhar & Petersen 2020), with previous work suggesting increased abundance of genera and species belonging to the phylum Pseudomonadota, and decreased abundance of beneficial *Bifidobacterium*. These antibiotic-induced perturbations also appear to affect host immunity and metabolism (Kwon *et al.* 2022).

An overall reduction in colonisation resistance, due to disturbances in the early-life gut microbiota, may

heighten risk of neonatal sepsis, which affects 3 million newborns each year. A neonatal observational study revealed that only a minority (13%) of patients receive WHO standard care of ampicillin and gentamicin for sepsis, and there is an increasing use of last-line agents such as carbapenems and polymyxin in some LMICs. Mortality at 28 days is high at 11.3%, and more than 59% deaths were due to hospital-acquired infections (Russell *et al.* 2023).

Carriage and transfer of MDR bacteria during early-life

Due to the rapid rise in AMR and MDR bacterial infections, there is a need to understand how different factors drive AMR, including MDR bacteria carriage and bacterial transmission routes (Andersson & Hughes 2017). Initially, vertical transmission of maternal microbes is one route by which the newborn may acquire microbes carrying AMR, particularly if the mother has received antibiotics during pregnancy (Li *et al.* 2021). Previous work has also shown that perinatal antibiotics also enhance horizontal gut colonisation (rather than vertical), which is defined as acquisition of strains from the environment (e.g. hospital settings), indigestion of food and interpersonal interactions. This may lead to carriage of potentially pathogenic strains (Li *et al.* 2021), due to antibiotic-induced gut microbiota perturbations creating a favourable niche for MDR bacteria from the (hospital) environment (Arboleya *et al.* 2022).

Within the hospital environment, infants are potentially exposed to a range of MDR bacteria. There are many examples of transfer of MDR bacteria between neonates including an outbreak of extensively drug-resistant *Acinetobacter baumanni* that involved 22 infants, with the strain introduced into the neonatal intensive care unit (NICU) via a colonised infant (Zarrilli *et al.* 2012). In Moroccan NICUs, 17% of newborns had acquired *A. baumannii* and its prevalence was 14% (Arhoune *et al.* 2019). Tunisian tertiary care evaluated MDR bacterial acquisition through nasal and rectal swabs, which indicated that *E. coli* was the most frequently detected bacterium on admission, with *E. coli* and *K. pneumoniae* detected on discharge. Length of stay, age and paediatric intensive care unit hospitalisation were risk factors for MDR bacterial acquisition during hospitalisation; with 9% paediatric patients acquiring at least one MDR strain (Tfifha *et al.* 2018). Thus, the hospital environment may play a crucial role in establishment of gut microbiota, and identification of resistant opportunistic pathogens is an important consideration for future infection risk and subsequent treatment options (Shao *et al.* 2019).

Livestock may also be a major contributor to resistance in animals and humans, and infants in LMICs with potentially closer contact with animals may lead to additional horizontal transmission of MDR bacteria (Rhouma *et al.* 2022). Furthermore, previous studies indicate that childcare contacts and family members may be a further reservoir of antibiotic resistance genes (ARGs); 77% of 80 healthy individuals' faecal samples have at least one ARG (Chen *et al.* 2022).

Horizontal gene transfer driving AMR in the early-life window

Although vertical transmission of mother microbes, and horizontal transfer of MDR strains from hospital environments, are major routes for establishing a 'resistome' in the gut microbiota of newborns, horizontal gene transfer (HGT) also plays a critical role in the development of AMR (Li *et al.* 2021). It is responsible for expanding the repertoire of ARGs between and within bacterial species (Jitwasinkul *et al.* 2016*b*). This complete 'resistome reservoir' of ARGs can be found in pathogenic and commensal bacteria, with movement of genes between microbes – including bidirectionally (Lerner *et al.* 2017, Appel & Vehreschild 2022). Indeed, a systematic study assessed the impact of 144 different antibiotics against 38 species of gut bacteria, and it showed β-lactamase resistance was common amongst gut commensals, likely facilitated via HGT (Maier *et al.* 2021).

Interestingly, high frequencies of potential HGT have been reported in infants' meconium and early faecal samples (Dimitriu 2022), indicating dynamic resistome development at early stages of microbial community structuring. Previous work also indicates that ARGs prevalence increases over time and during the first year of life (Loo *et al.* 2020*b*, Wintersdorff *et al.* 2016). Nogacka *et al.* found higher beta-lactam resistance in vaginally born Spanish infants whose mothers received IAP (Nogacka *et al.* 2017). Das *et al.* demonstrated that selected infant gut species (*B. longum* subsp. *infantis*, *Lactobacillus fermentum, L. gasseri* and *E. faecalis*) have more than 97% similarity with those in breast milk, and share similar ARGs abundances (Das *et al.* 2019).

However, it is important to note that these studies show associations rather than active HGT, and additional studies using, for example, Hi-C sequencing (as has been used in adult faecal samples) may allow for more accurate linkages with host bacteria strains and ARG carriage profiles (Yaffe & Relman 2020, Ivanova *et al.* 2022).

This ARG 'flow' depends upon the donor and recipient bacterium, and the type of mobile genetic element (MGE) driving HGT. Antibiotic susceptible bacteria may acquire resistance via a number of mechanisms including by mutations or MGEs such as integrons, transposons, bacteriophages and plasmids (Jitwasinkul *et al.* 2016*b*, Haudiquet *et al.* 2022). Among MGEs, conjugative plasmids are the major contributors for spread of AMR via transmission within and between bacterial species. However, a conflict exists for transmission of conjugative plasmids, as carriage can be associated with a reduction in 'host' fitness. Therefore, within a microbial community, e.g. the early-life gut microbiota, plasmid transmission is influenced by presence of susceptible hosts, which favours increased plasmid transfer. Moreover, there is a trade-off between the rates of vertical (i.e. passage of a plasmid from mother to daughter cells during division) and horizontal (i.e. passage of a plasmid from donor to any recipient cell outside of cell division, often through conjugation) plasmid transmission (Turner *et al.* 1998). When horizontal transmission increases, virulence of the receptor strain should also increase, thereby reducing the plasmid rate via vertical transmission (Dimitriu *et al.* 2021).

Conjugative plasmids mediate gene transfer in diverse environments. Their ability to donate F type conjugative plasmid R1 varies among enteric (i.e. gut) bacteria as sex-pili formations is repressed in bacterial strains with R1 plasmids. When plasmids encode virulence factors and ARGs, amplifier cells not only facilitate the emergence of new pathogenic strains but also affect the efficacy of antibiotic treatments. Thus, the identification of amplifier strains is of considerable importance for public health (Dionisio *et al.* 2002). In commensal *E. coli,* F plasmids are the most common conjugal plasmids, and they were the first to be associated with transmissible antibiotic resistance (Stephens *et al.* 2020). As *E. coli* is a core member of gut microbiota, particularly during early-life, further work is needed to probe factors that are involved in plasmid mediated HGT and AMR (Ott & Mellata 2022). Given the gastrointestinal tract has many different biofilm sites, this may also increase HGT of plasmids (via closer physical contact), and thus overall ARGs, which may contribute to over resistome profiles in the infant gut microbiota. Indeed, the expression of tetracycline and kanamycin genes has been found to increase three- to four- fold in biofilms of *E. faecalis* (Cook & Dunny 2013, Motta *et al.* 2021). Intriguingly, MGEs (plasmids) may also act as a barrier to HGT, as Lazdins and colleagues utilised a vector to displace resistance plasmid – IncP-1 plasmid RK2, as an alternative strategy to combat AMR (Lazdins *et al.* 2020).

ARGs can also be associated with integrons (non-mobile elements). There are five classes of integrons, with class 1 integrons the most widely studied. These are found in pathogens and commensals that contain different ARG cassettes conferring resistance to antibiotics (Loo *et al.* 2020*a*). In early-life, due to immaturity of the microbiota, and thus reduced colonisation resistance, this potentially provides a niche for exogenous bacteria carrying ARG integrons to efficiently colonise. Previous work has shown that integrase gene (*int1)* persistency was found throughout the first 2 years of life, including between mothers and their children, also indicating that maternal sources were possible routes for transmission. Additional transposons-containing integron genes on conjugative plasmids associated with sulphonamide, aminoglycoside and trimethoprim resistance similar to conjugative plasmid psH1148_107 were also detected (Ravi *et al.* 2015). Prophages (viral genomes integrated within a bacterial genome) can also media HGT and contribute to bacterial pathogenicity. Transduction can mediate resistance between bacterial species such as in *Enterococcus* by polyvalent phages (Chen *et al.* 2022). However, transfer of resistance genes by phages, particularly within the gut microbiota remains a complex subject (Borodovich *et al.* 2022).

Early-life AMR carriage

On average, it appears that the number of ARGs within infants increases with age, with all infants harbouring common ARGs (*blaZ, tet(M), fosA, lsa(A), erm(B)* and *aac(6′)-aph(2′′))* in the first year of life, which may correlate with increasing overall gut microbiota diversity (Loo *et al.* 2020*a*). Previous work has also detected high prevalence and persistence for the *int1* gene in infants at ages 3–10 days and 4 months, with persistency detected throughout the first 2 years of life between mothers and their children (Ravi *et al.* 2015). Over 9% of breastfed neonates born by caesarean section were found to have one or more ARGs within the first normal

faeces passed, with >85% of breast milk colostrum samples also containing ARGs, leading the authors to hypothesise ARGs may originate from breast milk and/or the environment (Zhang *et al.* 2022). In addition, a previous meta-analysis cohort study has also indicated that ARG load was higher in formula-fed infants, with the ARG load in premature formula-fed infants nearly double the load of breastfed infants. Formula-fed infants also have significantly more potential pathogens within the family Enterobacteriaceae, which are known to harbour many mobile ARGs (Pärnänen *et al.* 2021). Another study found that hospital environments, rather than the maternal microbiome, were the major sources of ARGs in neonates, with the first ARGs *bla*SHV and *mecA* only found in newborn samples rather than in maternal–infant pairs (Klassert *et al.* 2020). In addition, several studies have also shown a high prevalence of ARGs in infants who have had no prior antibiotic treatments, again suggesting a hospital (or maternal) environmental source (Ravi *et al.* 2015). This is highlighted by the similarity between microbes from the hospital environment and those found in the infants' gut (as discussed earlier). Such ARG interchange may lead to colonisation of pathogenic bacteria in neonatal wards including MDR bacteria (Klassert *et al.* 2020).

However, just using purely targeted ARG profiling approach(es) limits broader understanding on the carriage, mode of transfer, and bacterial strains involved; thus, additional approaches like in-depth metagenomics and culture are needed. Further phenotypic profiling is key, particularly for uncovering novel AMR genes and/or mechanisms (Andreoni, 2003, Qi *et al.* 2006, Sommer *et al.* 2009, Jitwasinkul *et al.* 2016*a*).

The many faces of *Enterococcus*

Many members of the gut microbiota can, in certain conditions, overgrow and cause serious infection in their host (Abt & Pamer 2014). This poses a significant problem in infant populations who are exposed to multiple microbiota perturbing factors and have an immature immune system, thus reducing their overall anti-infection responses. One such genus are *Enterococcus*, which comprise a diverse group of lactic acid bacteria (LAB) that can be isolated from different environments, but are commonly found in the gut of humans and animals. Different species and strains have been shown to act as 'beneficial' members of the gut microbiota, and there are some that are used as probiotics, particularly in the veterinary arena (Silva *et al.* 2012). Indeed, most enterococci are non-virulent, and have a low infection potential, with a recent study indicating that 4% of *Enterococcus* spp. isolated from humans showed mutualistic behaviour with a probiotic potential (Lohrasbi *et al.* 2020). This may be due to their ability to produce enterocins (enterococcal bacteriocins that are small antimicrobial peptides) that display broad-inhibitory spectrum activity against spoilage bacteria and foodborne pathogens such as *Bacillus cereus*, *Staphylococcus* spp., *Clostridium* spp. and *Listeria monocytogenes* (Ben Braïek & Smaoui 2019). Recently, a two-peptide leaderless bacteriocin produced by the *E. faecalis* 14 (strain previously isolated from meconium) named Enterocin DD14. These leaderless bacteriocins were discovered by Cintas and colleagues after characterisation of an enterococcal bacteriocin named L50. It is active wide range of Gram positive bacteria including MRSA, both *in vivo* and *in vitro*, including anti-adhesive activity (Ladjouzi *et al.* 2023, Pérez-Ramos *et al.* 2023). Furthermore, *Enterococcus* strains from breastfed infants showed higher inhibitory effects than those from adults, suggesting additional research in this area is required to further probe their probiotic potentials (Rahmani *et al.* 2020).

Crucially, there are a number of species and strains that are of significant clinical concern, including *E. faecalis,* accounting for 80–90% of enterococcal infections, and *Enterococcus faecium* which represents 5–10% (Silva *et al.* 2012). There is increasing concern due to increases in MDR *Enterococcus* strains, including vancomycin-resistant enterococci (VRE) which have been spreading steadily worldwide, and are common in long-lasting hospital outbreaks (Ramos *et al.* 2020). Moreover, given that *Enterococcus* strains can harbour vancomycin resistance encoded on plasmids, this increases risk of transfer to other susceptible *Enterococcus* species and strains, and also potential spread to other key clinical pathogens (and microbiota members) such as was found in MRSA (DeNap & Hergenrother 2005).

Mechanisms of AMR in *Enterococcus*

Intrinsic resistance (i.e. when a bacterial species is naturally resistant to a certain antibiotic without the need for mutation or gain of further genes) in *Enterococcus* is linked to an inability of aminoglycosides to enter the cell (where they inhibit ribosomal protein synthesis). However, at the population level, enterococcal

minimum inhibitory concentrations (MICs) have increased over time, which appears to link to increasing extrinsic resistance (i.e. acquiring resistance genes from other bacteria which are already resistance to antibiotics). Pheromone-sensitive, broad host range plasmids, and transposons drive HGT exchange among enterococci. Small peptide signals from potential recipients trigger expression of conjugative transfer genes in pheromone-responsive plasmids of *Enterococcus* (Zatyka & Thomas 1998). Broad host range plasmids transfer genetic information to *Enterococcus* species and strains and also other Gram-positive and even Gram-negative species (Hollenbeck & Rice 2012).

Three types of transposons (composite, conjugative and Tn3 transposons) are also responsible for genetic exchange (including ARGs) in enterococci. Prophylactic gentamicin is given to preterm infants as a treatment for potential bacterial infections (Shimizu *et al.* 2019), but high-level gentamicin resistance frequently occurs through *aph(2'')-la-acc(6')-le*, which is flanked by IS256 in composite transposon Tn5291 in *E. faecalis* (Hollenbeck & Rice 2012). This same gene is responsible for high-level resistance to all available aminoglycosides except streptomycin (Marothi *et al.* 2005). β-lactam and cephalosporin's use penicillin-binding proteins (PBPs) to disrupt cell wall synthesis that leads to production of reactive oxygen species. However, in enterococci these are removed by superoxide dismutase (Růžičková *et al.* 2020). Additional resistance is provided by *bla* genes (confer resistance through β-lactamases to β-lactam antibiotics) which can be transmitted between bacteria via plasmids.

Enterococcus strains can also encode acquired resistance to glycopeptides such as vancomycin. Although linezolid resistance is currently not common, it can be acquired through the *cfr* gene (alters adenosine in linezolid), which is encoded on transmittable plasmid pEF-01 (Růžičková *et al.* 2020). The gene *vanHBX* conferring resistance to vancomycin has been detected in *E. faecium,* and data suggests this can be acquired via phage transduction (Kondo *et al.* 2021). The *vanC* gene along with *vanRc, vanXYc* (which are protein homologs) were found in *Enterococcus gallinarum, Enterococcus casseliflavus and Enterococcus flavescens* confers intrinsic low level resistance to vancomycin (Clark *et al.* 1998, Younus *et al.* 2021). Vancomycin variants are found in these species including *E. faecium* as indicated in Fig. 1.

Enterococcal bacteriophages have previously been shown to play a role in transfer of ARGs in enterococci, as gentamicin (*ant2*-I-) and tetracycline *(tet(M))* were transferred within and between enterococcal species. However, although phenotypically resistant, none were detected by PCR (Mazaheri Nezhad Fard *et al.* 2011).

Why is it a problem clinically?

The name 'entero' specifies *Enterococcus* has an intestinal habitat, and indeed they have been found in high concentrations in adult human faeces - between 10^4 and 10^6 bacteria per gram wet weight (Boehm Ab 2014), although it is unclear what the levels are in infant faeces. These gut-associated strains in many cases will not be associated with infection, but they may act as a reservoir. Indeed, ARGs such as *tet(M))* and *erm(B)* have previously been shown to be encoded by *Enterococcus* spp. in healthy infants in a US-based study (Zhang *et al.* 2011). A recent EARS-Net (European Antimicrobial Resistance Surveillance Network) study showed that VRE caused around 16,000 nosocomial infections, and 1065 deaths in the EU/EEA in 2015 (twice the number reported in 2007). The burden of antibiotic-resistant associated infections in EU/EEA was highest in infants and people aged 65 years or older (Cassini *et al.* 2019, Murray *et al.* 2022). Current German data also highlights the same increasing trend (Markwart *et al.* 2019). Increasing VRE proportions in infants is of serious concern, as antibiotic resistance is associated with increase mortality and morbidity (Ayobami *et al.* 2020). *Enterococcus* infections, alongside *E. coli*, *Pseudomonas aeruginosa*, *Enterobacter* and *K. pneumoniae* are leading causes of hospital-associated bacteraemia, UTIs and endocarditis (Monegro Af & Regunatha 2022).

Crucially, gut-derived enterococci may act as a reservoir of opportunistic MDR strains that in certain circumstances may translocate to the bloodstream or other body sites and initiate serious infection, particularly in vulnerable patient groups like neonates (Dubin & Pamer 2017). Studies have shown that *Enterococcus* species (particularly *E. faecalis*) are one of the most dominant LAB colonising newborns (Al-Balawi & Morsy 2020), and preterm infants in particular appear to carry more *Enterococcus* in their gut as compared to full-term infants (Dahl *et al.* 2018). Linking to infections, *E. hirae* is a rare human pathogen, however a first paediatric case of catheter-associated bacteraemia in a 7-month-old infant has been reported (Brayer *et al.* 2019). Also, *E. faecalis* was found to be responsible for acute pyelonephritis in children aged 0–18 years at a German university tertiary care centre (Raupach 2019), and a NICU study reported a natural history of vancomycin-

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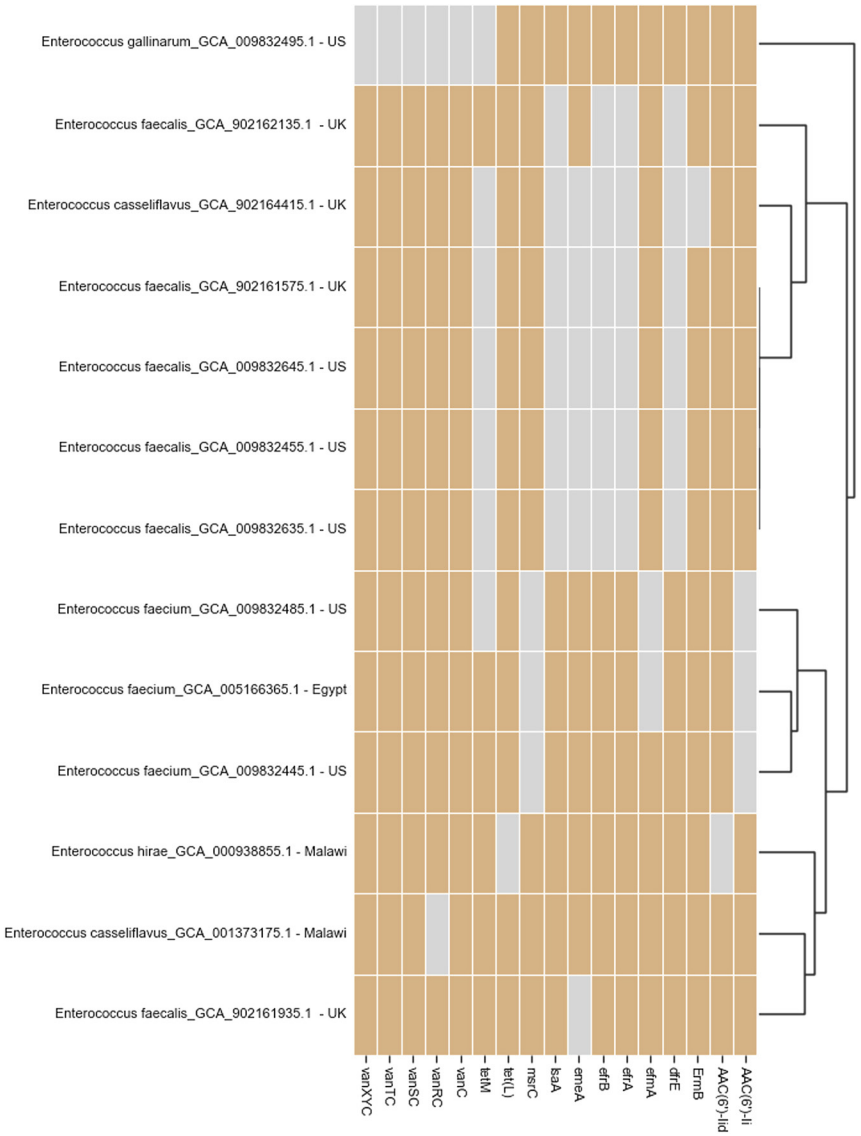

Figure 1
Presence and absence heat map for five different *Enterococcus* species (*E. faecalis, E. faecium, Enterococcus hirae, E. casseliflavus and E. gallinarum*) and strains from different countries indicating high prevalence of aminoglycoside, macrolide and glycopeptide genes. Heat map was generated using sea born package. Reference genomes used were obtained from NCBI (GCA_009832495.1, GCA_902162135.1, GCA_902164415.1, GCA_902161575.1, GCA_009832645.1, GCA_009832455.1, GCA_009832635.1, GCA_009832485.1, GCA_005166365.1, GCA_009832445.1, GCA_009832455.1, GCA_001373175.1 and GCA_902161935.1), with the CARD database used for prediction of AMR genes.

resistant *E. faecium* (VREF) carriage among infants after discharge from hospital (Schechner *et al.* 2022). MDR *E. faecium* and VREF have also been associated with neonatal sepsis, with the maternal gut traced as the source. This indicates monitoring of VRE colonisation in pregnant women should be undertaken to reduce neonatal sepsis occurrence (Subramanya *et al.* 2019).

How can we reduce carriage of potential AMR bacteria in the infant gut?

Current data indicates that infants who receive probiotics also receive less prescribed antibiotics, which may be linked to enhanced colonisation resistance (King *et al.* 2019). Previous studies have shown that *Lactobacillus* and *Bifidobacterium* strains reduce the risk of infection and reduce carriage of *Enterococcus* in the neonatal gut, this includes data showing that this probiotic supplementation reduces incidence of preterm-associated NEC and late-onset sepsis (which is associated with overgrowth of MDR bacteria including *Enterococcus*) (King *et al.* 2019, Robertson *et al.* 2020). This *Bifidobacterium* dominance, and lower abundances of pathobionts, more closely resembles a gut of a full-term infant (Alcon-Giner *et al.* 2020). Moreover, probiotic use of *Lacticaseibacillus rhamnosus* GG appears to enhance elimination of VRE in adults, preterm and newborn infants, potentially due to production of organic acids and/or bacterocins (Manley *et al.* 2007, Tytgat *et al.* 2016). Probiotic supplementation during pregnancy may also limit vertical transmission of potential MDR bacteria including *Enterococcus* to newborns, although

Table 2 Outstanding questions.

	Questions
1.	To what extent are antibiotic resistance genes in commensals a threat to human (infant) health?
2.	How does the maternal gut microbiome impact colonisation against AMR pathogen and/or infection?
3.	What are the actual HGT rates in the early-life gut – what mobile genetic elements are important, and is this driven by increasing intake of antibiotics?
4.	What is the carriage of *Enterococcus* and ARG determinants in healthy infants, and do these strains act as a reservoir for serious nosocomial infections?
5.	As *Enterococcus* may also be a normal member of the early-life gut microbiota, can we also mine this genus for development of new probiotics and as a source of targeted novel antimicrobials?

additional studies are required to more fully evaluate the role of probiotics during key early-life stages (Mueller *et al.* 2015).

As indicated above, formula feeding is correlated with a higher neonatal ARG burden, while breastfed infants appear to have less ARGs and also fewer diarrhoea associated hospitalisations (Pärnänen *et al.* 2021). The action of breast milk is likely combinatory; via transfer to pathogen specific IgA (in colostrum) and also provisions of HMOs that selectively feed beneficial bacteria like *Bifidobacterium,* which enhances colonisation resistance in the early-life gut (Kapourchali & Cresci 2020). Thus, increased breast feeding and/or access to donor breast milk banks may help to reduce carriage of MDR bacteria and also overall ARG within the wider microbiota (Nadimpalli *et al.* 2020).

The 'complete the antibiotic course' as dogma for reducing infections and AMR has been brought into question, with, for example, short antibiotic courses for pneumonia (5 days instead of 7 or 10 days) apparently equally effective as longer courses for uncomplicated infections (otitis media and streptococcal pharyngitis) (Langford & Morris 2017). Indeed, a BMJ study highlighted there is no evidence that stopping antibiotic courses early (or giving a shorter course overall) increases AMR, while taking for longer periods may increase the risk of resistance. Thus, improving antibiotic stewardship and bridging the clinical and public setting is key (Llewelyn *et al.* 2017). For infants, a recent study using antibiotic spectrum index (ASI), indicated new opportunities to improve antibiotic stewardship, and highlighted the utility for using this metric for comparing antibiotic exposures among NICU populations (Sullivan *et al.* 2022). However, work on these metrics to drive prescription changes, including linking to clinical outcomes, are required, and how this would work in broader community settings (Gerber *et al.* 2017).

Further strategies that are, and could be exploited, to prevent the emergence of AMR; include vaccines, new narrow-spectrum antimicrobials against drug resistant pathogens, and phage therapy (Micoli *et al.* 2021, Diamantis *et al.* 2022, Huang *et al.* 2022). Next-generation probiotics also have the potential to re-establish colonisation resistance, eliminate potential pathogens from the gut, and reduce antibiotic-resistant bacteria and their infections. This may also include mining the microbiota for strains that produce novel antimicrobials that target specific pathogenic taxa. Given, the significant interest in harnessing the microbiota as novel therapies, including those against drug-resistant infections, e.g. prevention of recurrence of *Clostridioides difficile* infection (CDI) in adults, recently approved by the FDA (REBYOTA from Ferring Pharmaceuticals), there is a huge scope for more targeted and personalised approaches (FDA, Ferring, 2 December /2022).

Conclusions and future perspectives

We face a clear and present danger related to AMR and associated overuse of antibiotics which has, and will continue to, significantly increase morbidity and mortality rates, particular in vulnerable infant populations. There are numerous open questions in this area that must be tackled using a multidisciplinary approach (Table 2). In particular, antibiotics disrupt the burgeoning early-life gut microbial communities which may also further exacerbate AMR and neonatal infections, including those caused by *Enterococcus* spp. Global and national action plans and improved awareness through effective communication, education and training for clinicians, the public and patients is key to counter the AMR challenge. However, there is a pressing need to address these issues in LMICs, given that children under 5 who died from AMR infections were nearly all (99.65%) from these resource-poor settings.

Insights into the gut resistome and impact of HGT on dissemination and carriage of ARGs are urgently

required, particularly within the dynamic early-life gut microbiota. The same 'niche' may also be harnessed for development of next-generation probiotics and novel antimicrobial strategies with additional studies in this area potentially providing new avenues for therapy development.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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