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Antibiotic resistance in the commensal human gut microbiota

Lisa E Lamberte and Willem van Schaik

Antibiotic-resistant infections are a major threat to global public health and there is an urgent need to develop new drugs and interventions to treat and prevent infections caused by antibiotic-resistant bacteria. The human gut microbiota harbours both commensals and opportunistic pathogens which can acquire resistance to antibiotics through mutation and horizontal gene transfer. The powerful combination of modern high-throughput DNA sequencing and microbiological culture methods is providing novel insights into the mechanisms of antibiotic resistance among, up to recently poorly studied, commensal bacteria in the gut. Interventions to minimise the abundance of antibiotic-resistant commensals and opportunistic pathogens include faecal microbiota transplantation and the use of live biotherapeutics, but the efficacy of these treatments remains elusive.

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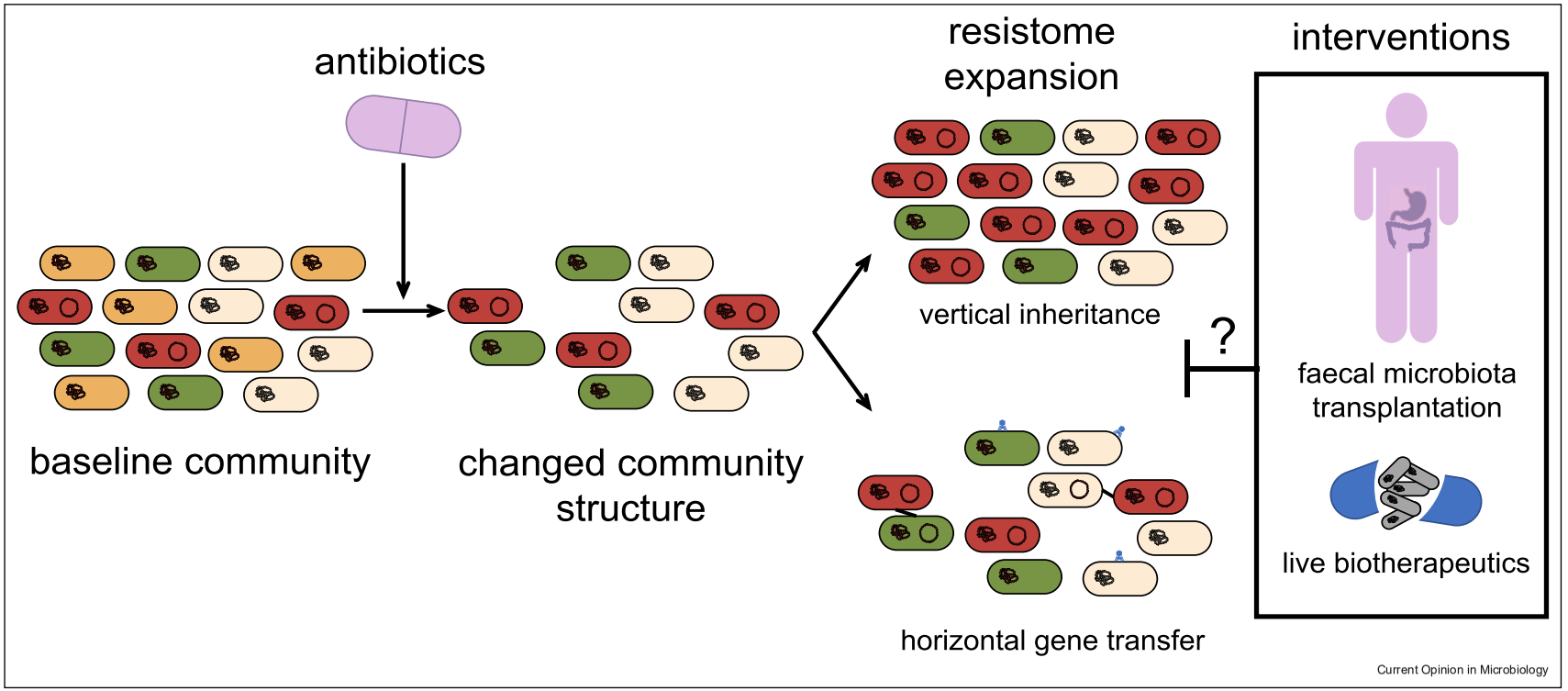
The human gut microbiota as a reservoir of antibiotic resistance genes

Antibiotic resistance is a grave threat to modern medicine and global public health. The global attributable mortality of antibiotic resistance is estimated to number around 1.3 million deaths [1]. Most of these deaths occur in low-income and middle-income countries, with the highest numbers in South Asia (an estimated 389 000 deaths) and Sub-Saharan Africa (approximately 255 000 deaths) [1]. Antibiotic resistance can be mediated by a variety of mechanisms, with the most important being the prevention of access of the antibiotic to its target, the prevention of the antibiotic binding to its target and the modification or degradation of the antibiotic [2]. Bacteria can develop resistance through mutations, which can then further spread vertically upon cell division to daughter cells. Alternatively, bacteria can acquire mobile genetic elements carrying antibiotic resistance genes in a process termed horizontal gene transfer (HGT). Additionally, genes transferred horizontally may also be inherited vertically from mother to daughter cells (Figure 1), further contributing to the spread of antibiotic resistance genes. Mechanisms of HGT include transformation, transduction, conjugation and DNA transfer via membrane vesicles [3]. Collectively, these pathways of HGT contribute to the global spread of antibiotic resistance genes in microbial ecosystems. The transfer of antibiotic resistance genes is predicted to be particularly high in ecosystems where microbial abundance and diversity are high, such as the bacterial communities found in the human gut [4•,5].

The term 'human gut microbiota' describes the collective of microbes that colonise the human intestinal tract [6]. Members of the gut microbiota typically have a symbiotic relationship with their human host, by supplying nutrients and providing protection from pathogenic organisms [7]. However, opportunistic pathogens can also be present in the gut microbiota. These are mostly carried asymptomatically in healthy individuals but can cause infections, particularly when the host is immunocompromised. These gut-dwelling, opportunistic pathogens include *Clostridioides difficile*, *Escherichia coli* and *Enterococcus faecium* [8–10]. In addition, species in the genus *Bacteroides*, in particular *Bacteroides fragilis*, are among the most prominent anaerobic causes of infection, even though they are widely regarded as commensal members of the gut microbiota [11,12]. Indeed, the human gut microbiota is now recognised as a reservoir of antibiotic resistance determinants, termed the 'gut resistome' [13,14]. Thus, there is an interest to study antibiotic resistance in the human gut microbiota and to characterise to what extent the resistome can contribute to the emergence of multidrug-resistant clones of opportunistic pathogens.

In this review, we discuss current research into the human gut resistome using high-throughput DNA sequencing and microbiological culture. We primarily focus on antibiotic resistance in gut commensals as resistance mechanisms of opportunistic pathogens that

Figure 1



Antibiotic resistance in the human gut microbiota. The gut microbiota contains a large diversity of bacteria, some of which can carry antibiotic resistance genes on mobile genetic elements, including plasmids (red cells). Upon exposure to antibiotics, susceptible strains are eradicated while strains with intrinsic or acquired resistance remain. Further expansion of the resistance gene reservoir can then occur due to vertical inheritance through cell division or HGT of mobile genetic elements. FMT and the use of live biotherapeutics are proposed to minimise colonisation by drug-resistant bacteria but their efficacy in achieving this goal is currently unclear.

colonise the human gut have been reviewed elsewhere [10,15,16]. We will also discuss current strategies to reduce the levels of antibiotic-resistant bacteria in the human gut by microbiota manipulation.

Antibiotic resistance in gut commensals

The human gut microbiota in healthy adults is a generally stable ecosystem that is mostly studied using high-throughput DNA sequencing. Initial studies showed that a large diversity of antibiotic resistance genes exists in the human gut microbiota with genes encoding resistance to tetracyclines, beta-lactams and macrolides being particularly prevalent across gut microbiotas of different individuals [17]. Methods to predict novel antibiotic resistance genes through machine learning approaches or on the basis of the predicted three-dimensional structure of the proteins they encode suggest that gut bacteria carry over 6000 uncharacterised antibiotic resistance determinants [18•,19]. With most high-throughput sequencing methods, it remains a challenge to link an antibiotic resistance gene to its bacterial host or hosts. This challenge can be overcome through the use of metagenomic chromosome confirmation capture methods, most prominently by a technique termed Hi-C, in which regions of bacterial DNA that are in close proximity to each other are captured and sequenced [4•]. When antibiotic resistance genes are thus linked to chromosomal markers, the microbial hosts of these antibiotic resistance genes can be identified. Studies using Hi-C have suggested that HGT of resistance genes is frequent in the human gut microbiota [4•]. The taxonomic resolution of Hi-C is, however, often insufficient to identify the hosts of resistance genes down to the strain- or species-level, and may not be able to identify links between resistance genes and chromosomal markers in members of the gut microbiota that are present at low abundance [20]. For this reason, the use of microbiological culture methods to isolate resistant bacteria from the gut microbiota is an essential alternative technique to profile the gut resistome, with high-throughput approaches being used to isolate, identify and characterise the microbial hosts of resistance genes [21].

A systematic study has recently assessed the impact of 144 different antibiotics on a set of strains representing 38 species of gut bacteria [22••]. This study showed that β-lactam resistance among gut commensals is strain-specific and most likely spreads through HGT. Remarkably, both macrolides and tetracyclines selectively kill a wide range of gut commensals, which suggests that these antibiotics may disproportionately affect the gut microbiota during therapy. As the species described in this study are representative to those of the healthy human gut microbiota, we use these bacteria as a basis to discuss some of the key members of the gut commensal community in the following paragraphs.

Among the quantitatively most prominent members of the gut microbiota, strains from the genus *Bacteroides* have high resistance rates across antibiotic drug classes, particularly β-lactams and tetracyclines [23–25]. Resistance to β-lactams in these genera is linked to the genes *cfxA*, *cfiA*, and *cepA*, while resistance to tetracyclines is linked to the gene *tetQ* [24]. The *tetQ* gene, along with the macrolide resistance gene *ermF*, is carried on a 65-kbp conjugative transposon, named CTnDOT, that is present in 80% of *Bacteroides* spp isolates. CTnDOT excision and conjugative transfer is triggered by exposure to low levels of tetracycline, thus leading to its rapid dissemination among *Bacteroides* strains [26].

The mucin-degrader *Akkermansia muciniphila*, a species in the phylum Verrucomicrobia, is near ubiquitously present in the adult human gut microbiota, making up approximately 3% of the bacteria in the colon [27]. Levels of *A. muciniphila* are lower in individuals with a variety of conditions, including obesity, metabolic syndrome and diabetes. This observation has led to research efforts to better understand the impact of *A. muciniphila* on human health [28]. Antibiotic resistance in *A. muciniphila* has so far been poorly studied. A recent study showed that the type strain of *A. muciniphila* was resistant to nearly all quinolone antibiotics [22••]. Genome sequence analysis of 39 *Akkermansia muciniphila* strains showed that a single strain among this dataset had acquired sulphonamide and aminoglycoside resistance genes through an HGT event from the *Salmonella enterica* plasmid pRSF1010 [29]. As *A. muciniphila* is proposed as a live biotherapeutic that could positively contribute to host health [30], a deeper understanding of its antibiotic resistance mechanisms and the potential of its resistance genes to spread horizontally is urgently needed.

Species in the genus *Bifidobacterium* are abundant in the infant gut, but present at lower levels in adult gut. Supplementation of infant nutrition with *Bifidobacterium* to promote health is a topic of considerable interest [31]. It is therefore important to ensure that probiotic *Bifidobacterium* strains are free of any relevant antibiotic resistance genes important to infant gut health. An exception can be made for the gene *ileS*, which confers intrinsic mupirocin resistance [32]. In a study that assessed the resistome of *Bifidobacterium* species from gut metagenome data sets of adults and infants, the tetracycline resistance gene *tetW* was predicted to be carried on a conjugative transposon, suggesting they can be acquired or disseminated via HGT [33].

C. difficile has been shown to develop resistance to metronidazole through the acquisition of a high-copy

plasmid, presumably via HGT, although the donor could not be identified [34]. HGT in the gut microbiota appears to be particularly prominent among members of the phylum Firmicutes and it is thus likely that there is widespread sharing of resistance genes in this phylum, including between commensals and opportunistic pathogens, like *C. difficile* and *Enterococcus* [35••].

However, it is still unclear to what extent antibiotic resistance genes in commensals are a threat to human health. An important recent study set out a framework that can be used to assess the risks associated with finding resistance genes in microbial genome sequences [36•]. The resistance genes in the highest risk category are those that are associated with mobile genetic elements in human pathogens. The large majority (81.6%) of antibiotic resistance genes were, however, not associated with mobile genetic elements. These genes are thus likely to be intrinsic determinants of antibiotic resistance, and, if found in a commensal, may not meaningfully contribute to the burden of resistance genes among pathogens.

Interventions to reduce expansion of the gut resistome

The human gut resistome is affected by a variety of factors, including changes in diet and exposure to antibiotics [37,38]. In particular, members of the families *Enterobacteriaceae* and *Enterococcaceae*, which contain several opportunistic pathogens, were found to expand and persist during antibiotic treatment of healthy adult individuals [39,40]. Additionally, antibiotic treatment diversifies the resistome across individuals, suggesting that the resistome composition is highly individualised [37]. The impact of the resistome is well recognised in vulnerable individuals such as preterm infants and immunocompromised individuals. In particular, the gut microbiota in hospitalised patients undergoes rapid and dynamic changes, such as the loss of microbial diversity and the expansion of opportunistic pathogens, during their stay [41,42]. However, commensal anaerobes present during admission may prevent the expansion of pathogenic bacteria by suppressing their growth and colonisation [42,43].

Given the importance of the resistome in human health, methods to circumvent the expansion of the resistome by manipulating the microbiota are being explored. Currently, the most-studied methods include faecal microbiota transplantation (FMT), and the use of live biotherapeutics (probiotics) which could suppress the outgrowth of bacteria carrying antibiotic resistance genes (Figure 1). Of these, FMT is the most dramatic intervention to modulate the composition of the gut microbiota as it involves a replacement of the original gut microbiota of the host by new microbiota provided via the stools of a healthy donor [44]. FMTs are effective in the treatment of recurrent *C. difficile* infections and FMTs in patients suffering from *C. difficile* infections have been shown to lead to the elimination of antibiotic resistance genes from the gut microbiota as well [45]. The success of FMT in the treatment of *C. difficile* infections has spurred several studies into the use of FMT to eradicate gut colonisation by multidrug-resistant bacteria. The authors of a recent systematic review on a total of 36 studies covering 254 patients remarked that variability in patient populations, FMT protocols and the multidrug-resistant bacteria that are being targeted by the treatment complicate the interpretation of data on the efficacy of FMTs on gut colonisation by multidrug-resistant bacteria. Despite these limitations, however, FMT was associated with a moderate amount of reduction (ranging from 20% to 90%) of gut colonisation by multidrug-resistant bacteria [46•].

Evidence for the effectiveness of probiotics as a method for microbiota recovery and eradication of antibiotic-resistant bacteria after antibiotic exposure has so far been elusive [47]. Studies using lactic acid bacteria, which are traditionally used in probiotic products, even suggested that probiotics might negatively affect the reconstitution of the gut microbiota post-antibiotic exposure [48]. A recent study determined the impact of taking both antibiotics and probiotics (a commercially available probiotic supplement composed of 11 strains from the *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, and *Lactococcus* genera) on the human gut resistome. In individuals where the probiotics colonised the gut, and which were not treated with antibiotics, a reduction in antibiotic resistance gene load was observed in an individual-specific way [49•]. Currently, there is significant interest in the use of novel live biotherapeutic products which contain gut commensals that have not been traditionally used as probiotics in food products [50], but no studies have so far studied the impact of these novel products on the levels of antibiotic resistance genes in the gut microbiota [47]. The use of novel live biotherapeutic products can, however, be an intriguing approach as recent studies in gut microcosms suggest that the gut microbiota can suppress growth of *E. coli* and its evolution towards antibiotic resistance upon exposure to an antibiotic [43]. Other promising interventions include prebiotics, postbiotics, phage-mediated therapies, conjugation inhibitors, and vaccines, although only a limited number of studies that has been conducted with inconsistent outcomes [47,51]. Finally, it may be possible to redeploy existing drugs to protect commensal bacteria against exposure to antibiotics. While the precise mechanisms of these antidotes have not been characterised, the anticoagulant dicumarol provided some protection to *Bacteroides vulgatus* upon exposure to erythromycin in a mouse model [22••].

Conclusions

The spread of antibiotic-resistant pathogens is a major public health concern and there are ongoing intensive research efforts to develop new drugs and interventions to treat and prevent multidrug-resistant infections. As commensals in the human gut microbiota frequently carry antibiotic resistance genes, these bacteria may contribute to the emergence of resistant clones of opportunistic pathogens, particularly in the rare events where HGT occurs across phylogenetic barriers [35••,52]. For this reason, there is an interest to develop interventions that reduce the selection for antibiotic-resistant bacteria in the human gut microbiota (Figure 1). Both FMTs and the administration of live biotherapeutics have shown some promise to reduce carriage of multidrug-resistant bacteria in the gut, but significant variations in the success of these interventions have been observed. Due to the complex interactions between the host, the microbiota, and external factors (e.g. diet), it may be unlikely that there will be an easy 'one-size-fits-all' solution to reduce the burden of antibiotic-resistant bacteria in the gut microbiota. Despite this observation, there remains an urgent need for studies on unravelling the role of gut bacteria in the dissemination of antibiotic resistance genes to opportunistic pathogens. Insights from these studies can be useful to identify novel 'hubs' of resistance gene dissemination among commensal bacteria, potentially leading to the development of targeted approaches to inhibit HGT or to eradicate these strains from the gut.

Conflict of interest statement

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: W.v.S. has received consultancy fees from Vedanta Biosciences.

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