The Gut Microbiome as a Reservoir for Antimicrobial Resistance

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Abstract: This review will discuss the gut as a reservoir for antimicrobial resistance, colonization resistance, and how disruption of the microbiome can lead to colonization by pathogenic organisms. There is a focus on the gut as a reservoir for *β*-lactam and plasmid mediated quinolone resistance. Finally, the role of functional metagenomics and long read sequencing technologies to detect and understand antimicrobial resistance genes within the gut microbiome, and the potential for future microbiome-directed methods to detect and prevent infection is discussed.

Keywords: Microbiome, resistome, antibiotic resistance, antimicrobial resistance

Introduction/Background

Antimicrobials transformed the practice of medicine, but from the moment antimicrobials were discovered, their effectiveness was compromised by the emergence of antimicrobial resistance (AR)[1]. AR can be encoded for on antibiotic resistance genes (ARG), or antibiotic target mutations. These mutations may be intrinsic, or disseminated through microbial communities via vertical inheritance, or horizontally via mobile genetic elements (MGE) and extrachromosomal plasmids. Historically, AR was predominately described in pathogens isolated from people with clinically significant infection. It is now known that AR can reside in organisms isolated from the microbiomes of asymptomatic people, and can later lead to infection when specific conditions create a permissive niche for the organism to contribute to disease[2].

The gut is a prime reservoir for AR organisms. The healthy gut microbiome is a stable, diverse community which provides important benefits to the host such as nutrient acquisition and protection from pathogens. Antibiotics can perturb this ecosystem by changing its taxonomic and functional composition, creating opportunities for pathogen colonization[3]. This “dysbiosis” can allow for AR colonization, increased ARG burden, and enable subsequent AR pathogen invasion into the blood stream, urinary tract, and other organ systems[4]. Thus, it is becoming increasingly important to understand how dysbiosis can drive AR in the gut microbiome, and how to prevent or reverse dysbiosis.

Metagenomic analysis of the gut microbiome is rapidly expanding our knowledge of AR, uncovering an incredible diversity of AR genes and plasmids which can be transferred to other organisms within the gut[5]. With rapidly expanding efforts to develop microbiome directed diagnostics and therapeutics, there is a need to characterize and quantify the role of the gut as a reservoir for ARG

carriage and exchange. Functional metagenomics is a culture-independent approach to uniquely characterize both known and uncharacterized ARGs. Functional metagenomics and can serve as both a discovery engine for cryptic and emerging AR, as well as to model the risk of horizontal gene transfer of AR[6]. The recent expansion of long-read sequencing (LRS) technologies offers a powerful complement to functional metagenomics, as it can associate ARGs with their host bacteria and mobilization elements, enabling accurate estimations of how ARs are exchanged between bacteria[7]. In concert with microbiologic culture, these culture-independent technologies hold the potential to improve our understanding of AR.

This review will discuss how "dysbiosis" of the microbiome can lead to gut colonization by pathogens, increased AR, and its role as a reservoir for β-Lactam and plasmid mediated quinolone resistance (PMQR) resistance. This review will also discuss applications of functional metagenomics and LRS to detect and understand ARGs and transmission within the gut microbiome, and the potential for future microbiome-directed methods to detect and prevent infection.

Colonization resistance and disruption

The healthy gut microbiome is a complex, diverse community which is resistant to colonization and proliferation by pathogens[8]. It is thought that "colonization resistance" occurs either through direct competitive interactions between bacteria, or indirectly through commensal bacteria triggering a host response against pathogens[9]. Antimicrobials has been shown to cause disruptions to the gut microbiome by lowering the bacterial diversity of the gut microbiome and thereby allowing pathogens to invade[10]. Once diversity has been compromised it can be difficult to ameliorate[10].

The gut as an AR reservoir

Gut commensal organisms have been previously thought to be innocuous, however breakthroughs in sequencing technology and techniques for determining function and transfer capability are revealing a more nuanced picture of the role of commensals in the gut resistome[11]. Mobile elements such as plasmids can be readily shared between commensal and pathogenic species[12]. Here we will discuss, β-lactam and plasmid-mediated quinolone resistance because of their propensity to be located on MGEs facilitating their spread, their ubiquity of β-lactam and Quinolone use around the world, and the importance of the aforementioned antibiotics as essential treatment options for many different types of bacterial infections.

The gut as a reservoir of* β*-lactam resistance

β-Lactams are the most commonly prescribed antibiotic class worldwide[13]. Microbiomes from 30,000 year old permafrost revealed enzymes within the TEM family which confer resistance to β-lactams[14]. TEM β-lactamases are a family of enzymes which are often located on plasmids and confer resistance to early cephalosporins and penicillins[15]. β-lactamases can be easily spread, with evidence of transmission of these resistance elements from one location to another, with humans serving as vectors by travelling. In one study 12/18 Swedish students tested negative for ESBL-producing bacterial isolates in the gut microbiome before travel, but later tested positive for ESBLs after travel to India[16].

Widespread range and transmission of ESBLs via plasmids has been identified, with community-associated ESBL infections in the US accounting for over 1/3 of total ESBL infections[17]. *Faecalibacterium prausnitzii* and *Prevotella copri* isolated from fecal samples of healthy adults was found to be resistant to the cephalosporins ceftriaxone and cefotaxime[18]. Metagenomic analysis

of the sequenced isolates found that many of their AR genes were located near mobilization elements such as integrases or on plasmids, indicating evidence of gene transfer.

The gut as a reservoir of PMQR

The primary method of resistance to quinolones arises in the form of single nucleotide polymorphisms (SNPs) located in areas termed quinolone resistance-determining regions, but the last few decades have revealed a new method of quinolone resistance: PMQR[19]. Travel to an area of high endemic resistance can act as a vector for transmitting PMQRs; travelers from The Netherlands were found to have significant acquisition of PMQRs after returning from Southeast Asia and India[20]. Phylogenetic studies of this family of enzymes confirmed that PMQRs can be found in soil microbiomes and the gut microbiomes of chickens and humans, suggesting an ecological niche to which it is endogenous[21].

It should be noted that there is an important distinction between species which have intrinsic versus acquired resistance[22]. Many important Gram-positive gut commensals are intrinsically resistant to quinolones, and acquisition of quinolone resistance can occur in commensal *E. coli* after antimicrobial exposures[23]. Recent work has elucidated more about the origins of quinolone resistance in the gut microbiome. The chromosomal ancestral source of *qnrB* is theorized to be *Citrobacter*; 37 *Citrobacter freundii* isolates from a Massachusetts hospital contained only *qnrB*, with only two showing the ability to transmit this resistance through conjugation[24]. There are several *Citrobacter* commensals in the gut, suggesting that it may be an endogenous reservoir for low level quinolone resistance. There still remains much to learn about the range of the AR reservoir in the microbiome, and its origins.

Enhanced methods to investigate the AR reservoir

The gut is host to many bacterial species which are challenging to culture via traditional microbiologic methods, making it difficult to investigate their contribution to the AR reservoir[11]. Functional metagenomics is a high throughput culture independent approach to assay the functional activities of microbial communities, enabling the functional genetic surveillance of difficult to culture organisms[11, 25]. In functional metagenomics, the total microbial community DNA is transformed into a culturable indicator strain (e.g., *E. coli*) which is then phenotypically screened for acquired resistance to different classes of AR. Through this method, functional metagenomics can model mobilizable AR risk by estimating the resistance elements that can be functionally utilized by an organism such as *E. coli*. For each organism of interest, large amounts of genetic material can be simultaneously assayed, and acquired phenotypic resistance profiles generated. Importantly, this technique does not rely on novel AR genes sharing sequence identity to known AR determinants. A research group functionally validated over 1000 AR genes from fecal and environmental, over 10% of which were novel[26].

Functionally validating AR genes unlocks a better understanding of the AR reservoir, but does not identify the original bacterial host. Thus, a complementary method is needed to characterize the broader genomic context of AR in the microbiome and identify the greatest clinical threats. Surveying and identifying AR in the gut microbiome has primarily been accomplished with "short-read" sequencing (SRS)[11]. These short reads (<500 base pairs) are generally insufficient to assemble circular contigs which can distinguish between chromosomal and plasmid DNA, though recently developed technologies can identify integration of other types of MGE[27, 28]. New technologies utilize long read sequencing (LRS) which can generate reads of 10's of kb in length. These fragments are able to resolve repetitive regions and generate high quality reference assemblies[29].

Both LGS and functional metagenomics, and especially a synergy of the two techniques, enable unparalleled insight into context and function of the AR reservoir. They are an invaluable resource as we move towards a future of microbiome-directed methods of identifying and preventing the spread of AR and infection.

Mobile Genetic Elements proliferate AR in the gut

Functional metagenomic analysis provides evidence that AR genes in pathogens are more frequently co-localized with mobility elements than AR genes in environmental microbiomes[30]. A recent report interrogating the resistome in wild and captive gorillas, chimpanzees, and co-localized humans found AR genes near MGEs with high sequence similarity from all three sources[31]. These data suggest that the microbiomes of wild and captive animals may be important reservoirs of AR. In another study, Bertrand *et al.* applied a hybrid sequencing approach using SRS and LRS to gut microbiome samples, enabling assembly of species genomes from the metagenomes of patients who underwent antibiotic therapy[7]. They discovered multiple plasmids unknown to the medical community, and new regions of multi-drug resistance within bacterial species; among these were multiple combinations of carbapenemases co-occuring with ESBLs[32]. One new region conferred resistance to carbapenems, aminoglycosides, trimethoprim, and sulfonamides. Previously, this region was not able to be assembled by SGS due to repeat regions, highlighting the opportunity LRS provides to investigate the AR reservoir of the gut microbiome.

LRS is also creating new opportunities to investigate understudied vectors of AR. A recent work identified two new megaplasmids (>420kb) carried by *Pseudomonas aeruginosa* clinical isolates harboring a shared core genome and varying AR gene carriage. GenBank homology searches

revealed 72 more bacteria harboring similar megaplasmids, isolated from all over the world, and as far back as 1970.

Utilizing both functional metagenomics and LTS can reveal nuanced and even more interpretable relationships between AR and the microbiome. In a remarkable study Kintses *et al.* used functional metagenomics to describe the reservoir of antimicrobial peptide and AR genes, then used LGS to contextualize genes to mobile elements[33]. Their investigation revealed that phenotypic resistance in *E. coli* via AR is much more likely to be successfully transferred, located on mobilizable genetic elements, and has fewer phylogenetic barriers to transfer. This is an interesting finding given that the gut microbiota is a known reservoir of antimicrobial peptide genes, and their prevalence was similar to AR genes after selection[34].

Clinical Implications

Insight from investigations into the reservoirs of AR have thus far been relegated mostly to the academic sphere of medical influence but holds promise for integration into clinical practice. New methods are being assimilated into established metagenomic pipelines, increasing the ease-of-use and potential incorporation into clinical practice[35, 36]. The most obvious and immediately useful clinical application is in investigation of structural variants and resistance genes in outbreak tracing, in which horizontal transfer is increasingly found to play a key role in AR transmission[37-40]. Furthermore, there are avenues for these techniques to be eventually used in personalized medicine, such as the recently developed Microbiome-Derived-Metabolism screen which produces an *ex-vivo* exploration of drug-microbiome interactions for individual patients[41]. This technique identified enzymes capable of metabolizing hydrocortisone within the human gut microbiome

through functional metagenomic screens, a feat unable to be accomplished with deep sequencing alone.

Future Directions and Conclusions

The pace of AR is quickly outpacing the discovery of new antimicrobials, thus continued research into the gut microbiome as an AR reservoir is also of utmost importance. Key areas for future investigations include 1) Clinical and translational studies delineating the features in the gut microbiome that are permissive to, or protective against, gut colonization with AR organisms. To achieve this, long-term follow up of asymptomatically colonized people is needed to assess the risk for progression to infection. 2) Characterization of MGEs within the microbiome, and a greater survey of their carriage in both pathogens and commensals. 3) Utilization of functional metagenomic to identify pathogens that are traditionally difficult to grow via microbiologic culture to identify the relationship between ARGs and the gut microbiome. 4) Studies utilizing long-read sequencing to leverage their potential to offer almost real-time analysis of sequencing data. 5) Metagenomic methods to rapidly identify AR profiles for clinical use. These are future avenues for study that could lead to direct improvements in patient care by providing rapid methods of pathogen identification and characterization.

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