The Journal of Infectious Diseases

SUPPLEMENT ARTICLE

The Gut Microbiome as a Reservoir for Antimicrobial Resistance

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This review will consider 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 is discussed, along with the potential for future microbiome-directed methods to detect and prevent infection.

Keywords. Microbiome; resistome; antibiotic resistance; antimicrobial resistance.

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 (ARGs) or antibiotic target mutations. These mutations may be intrinsic or disseminated through microbial communities via vertical inheritance or horizontally via mobile genetic elements (MGEs) 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 that 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 enable AR colonization, increased ARG burden, and 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.

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The Journal of Infectious Diseases® 2021;223(S3):S209–13

DOI: 10.1093/infdis/jiaa497

Metagenomic analysis of the gut microbiome is rapidly expanding our knowledge of AR, uncovering an incredible diversity of ARGs 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. It can serve as both a discovery engine for cryptic and emerging AR, as well as a way 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, because 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. It 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 that 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 have 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, but 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 PMQR, because of their propensity to be located on MGEs facilitating their spread, the 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 that 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 of 18 Swedish students tested negative for extended-spectrum β-lactamase (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 United States, accounting for more than one-third 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 ARGs 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 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 that have intrinsic resistance and those with acquired resistance [22]. Many important gram-positive gut commensals are intrinsically resistant to quinolones, and acquisition of quinolone resistance can occur in commensal *Escherichia 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 2 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 that are challenging to culture using standard 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 (eg, *E. coli*), which is then phenotypically screened for acquired resistance to different classes of AR. With this method, functional metagenomics can model mobilizable AR risk by estimating the resistance elements that can be functionally used 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 ARGs sharing sequence identity to known AR determinants. A research group functionally validated >1000 ARGs from fecal and environmental samples, >10% of which were novel [26].

Functionally validating ARGs unlocks a better understanding of the AR reservoir, but it 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 [11]. These short reads (<500 base pairs) are generally insufficient to assemble circular contigs that can distinguish between chromosomal and plasmid DNA, though recently developed technologies can identify integration of other types of MGE [27, 28]. New technologies use LRS, which can generate reads tens of kilobases in length. These fragments are able to resolve repetitive regions and generate high-quality reference assemblies [29].

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

MGE PROLIFERATION OF AR IN THE GUT

Functional metagenomic analysis provides evidence that ARGs in pathogens are more frequently colocalized with mobility elements than ARGs in environmental microbiomes [30]. A recent study interrogating the resistome in wild and captive gorillas, chimpanzees, and colocalized humans found ARGs near MGEs with high sequence similarity from all 3 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 [7]. applied a hybrid sequencing approach using short-read sequencing and LRS to gut microbiome samples, enabling assembly of species genomes from the metagenomes of patients who underwent antibiotic therapy. They discovered multiple plasmids unknown to the medical community, and new regions of multidrug resistance within bacterial species; among these were multiple combinations of carbapenemases occurring together with ESBLs [32]. One new region conferred resistance to carbapenems, aminoglycosides, trimethoprim, and sulfonamides. Previously, this region could not be assembled by short read sequencing owing 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 [32] identified 2 new megaplasmids (>420 kilobases) carried by *Pseudomonas aeruginosa* clinical isolates harboring a shared core genome and varying ARG carriage. GenBank homology searches revealed 72 more bacteria harboring similar megaplasmids, isolated from all over the world, and as far back as 1970.

Using 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 [34] used functional metagenomics to describe the reservoir of antimicrobial peptide and ARGs, and they then used LRS to contextualize genes to mobile elements. Their investigation revealed that phenotypic resistance in *E. coli* via AR is much more likely to be successfully transferred and located on mobilizable genetic elements, and it 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 the prevalence of these genes was similar to that of ARGs after selection [34, 35].

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 [36, 37]. 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 [38–41]. 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 [42]. This technique identified enzymes capable of metabolizing hydrocortisone within the human gut microbiome through functional metagenomic screens, a feat that cannot be accomplished with deep sequencing alone.

FUTURE DIRECTIONS AND CONCLUSIONS

Because the pace of AR is quickly outpacing the discovery of new antimicrobials, continued research into the gut microbiome as an AR reservoir is also of utmost importance. Key areas for future investigations include the following: (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 in 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) use 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 using LRS to leverage their potential to offer almost real-time analysis of sequencing data; and (5) metagenomic methods to rapidly identify AR profiles for clinical use. These are all future avenues for study that could directly improve patient care by providing rapid methods to identify and characterize pathogens.

Notes

Disclaimer. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Financial support. This work is supported by the National Institute of Allergy and Infectious Diseases, National Institutes of Health (grant 1K23AI137321 to J. H. K.). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Supplement sponsorship. This work is part of a supplement sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health (NIH) and the Centers for Disease Control and Prevention (CDC).

Potential conflicts of interest. All authors: No potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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