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The Threat of Antimicrobial Resistance on the Human Microbiome

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Abstract

Ubiquitous in nature, antimicrobial resistance (AMR) has existed long before the golden age of antimicrobials. While antimicrobial agents are beneficial to combat infection, their widespread use contributes to the increase and emergence of novel resistant microbes in virtually all environmental niches. The human microbiome is an important reservoir of AMR with initial exposure occurring in early life. Once seeded with AMR, commensal organisms may be key contributors to the dissemination of resistance due to the interconnectedness of microbial communities. When acquired by pathogens however, AMR becomes a serious public health threat worldwide. Our ability to combat the threat of emerging resistance relies on accurate AMR detection methods and the development of therapeutics that function despite the presence of antimicrobial resistance.

INTRODUCTION

Antimicrobial resistance (AMR) is a global health concern of increasing magnitude [1]. While clinical settings have traditionally been the main focus of the emergence of AMR, non-clinical environments are becoming increasingly recognized as an important factor in the dissemination of antimicrobial resistance genes (ARGs). We are now aware that anthropogenic, commensal, and environmental microorganisms all contribute to the reservoir of ARGs collectively forming the antibiotic resistome [2]. The selection pressure driven by the use and misuse of antimicrobials in prescribed human medicine and in disease prevention, control, treatment, and improved growth rates in food-producing animals has significantly contributed to this phenomenon. This has been compounded by the transmission of resistant microbes from person to person and from environmental sources, all influencing the spread of AMR. The microorganisms that inhabit the human body, the human microbiome, and its susceptibility to the spread of AMR, is of particular importance to human health. This increased vulnerability is not only a deadly threat for humans but also constitutes a mobile resistome capable of extending AMR among human populations

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worldwide. As new resistance mechanisms emerge and spread globally, this will continue to be exacerbated.

Antimicrobial agents have been used for decades to treat infectious disease, which has successfully reduced illness and death from infectious microbial species. It has become well recognized that microorganisms including bacteria, fungi, parasites, and viruses are capable of developing resistance that make these agents less effective [3–8]. According to the Centers for Disease Control and Prevention (CDC), in the United States alone, an estimated 2 million people become infected with antibiotic resistant bacteria, resulting in at least 23,000 deaths each year as a direct result of these antibiotic resistant infections [1]. Similarly, in Europe, an estimated 25,000 deaths are attributable to antibiotic-resistant infections annually. Global prevalence of azole resistance in *Aspergillus* is estimated to be 3–6%, and approximately 7% of all *Candida* bloodstream isolates are resistant to fluconazole [8], with echinocandin resistance on the rise [3]. According to the World Health Organization (WHO), in 2015, artemisinin resistance was confirmed in 5 countries of the Greater Mekong subregion along with the first signs of emerging multidrug resistance to all available antimalarial medicines. While resistance mechanisms are emerging in all groups of microbes, an important root cause contributing to this increase is the global over- and misuse of antibiotics. As such, this review focuses on the mechanisms that define the development, transfer, and subsequent detection of bacterial resistance in the human microbiome with an emphasis on the impact of AMR in human health. The emergence of AMR is superseding our ability to combat infection and the development of novel antibiotic therapies is essential to address this growing threat.

Historical perspective of emerging antimicrobial resistance

The advent of antimicrobials during the 20th century revolutionized medicine by controlling the spread of infectious disease. However, not long after the modern antimicrobial era began did warning signs of emerging microbial resistance to some of these compounds surface, turning what was the golden age of antimicrobials into a major health crisis. Antibiotics have been long used throughout human history as ancient folk medicine, with some of the earliest traces of tetracycline found in skeletons of Sudanese Nubia (A.D. 350–500) [9]. For as long as antibiotics have existed, resistance mechanisms have coexisted. Well before the antibiotic era and influence of anthropogenic drugs, ARGs have been identified in the microbiota of an 11th century mummy [10], 30,000 year old permafrost sediment [11], and a four million year old isolated cave [12]. Antimicrobials and resistance mechanisms are abundant in all microbial communities having likely evolved as important evolutionary and regulatory functions [13].

The trend towards administering high doses of broad spectrum antibiotics in response to infection, coupled with the wide use of antibiotics in commercial animal production systems have resulted in the generation and spread of ARGs. Genetic mutations for example can be induced by inter- or intra-species competition, exogenous antibiotic pressure, and immune recognition and response. Furthermore, ARGs can be acquired by genetic recombination through horizontal gene transfer (HGT), conjugation, phage transduction, or transformation [14, 15]. Conjugation describes genetic material being transferred from cell to cell, and is

usually facilitated by plasmids that often carry functional genes as well as their own replication machinery. Transduction is the result of acquiring genetic material from a virus, either because the virus codes for the gene, or packaging errors cause the virus to deliver non-viral DNA. Finally, transformation is the ability of an organism to take in exogenous DNA from the environment and incorporate it into its own genome. These methods of passing genetic material from one organism to another are difficult to detect outside carefully controlled lab experiments, making estimation of their prevalence in nature extremely tenuous. Many ARGs including beta-lactamases can be found on plasmids carried by members of the Enterobacteriaceae, but the other forms of HGT have been more difficult to detect.

Following the discovery and wide use of penicillin, plasmid-mediated penicillin-resistant *Staphylococcus* emerged and studies were undertaken to counter the increasing problem of penicillin-resistance. Methicillin, an alternative form of penicillin, was introduced and not long thereafter methicillin-resistant *Staphylococcus aureus* (MRSA) was identified [16]. Today, MRSA is resistant to an entire class of antibiotics, and like other microbial pathogens that acquire multiple resistance traits over time [17–20], has evolved multidrug-resistant variants [17–19]. These super-resistant strains are often associated with increased virulence and transmission characteristics thereby enhancing morbidity and mortality as the effectiveness of antimicrobials as therapeutic agents against these strains diminishes due to increased resistance [21].

The human microbiome as a reservoir for antimicrobial resistance genes

One important reservoir of AMR is the human microbiome, a complex ecosystem consisting of trillions of microbes closely interacting and exposed to resistance determinants. It is estimated that the human microbiome harbors about 3.3 million non-redundant genes, which represents a gene set 150 times larger than that of the human host [22, 23]. This extensive genetic diversity, along with the 10–100 trillion microbial cells making up this microecosystem, extend the host genetic, metabolic and immune capabilities primarily through regulating energy metabolism [24] and helping to shape innate and adaptive immunity [25]. The confined locations of this high cell and genetic density also provide ideal conditions for genetic exchange between transient and residential microbes, and among residential microbes. For instance, the human distal gut harbors about 10^{14} microbial cells representing around 400 different bacterial phylotypes [26, 27]. While the GI microbiome harbors commensal organisms, it also contains undesirable genetic traits linked to microbial virulence [28] and AMR [29], and these traits can be spread from organism to organism by HGT.

Estimates of HGT across different sites in the human microbiome point to over 13,500 events of HGT in only 300 species [30] identified through whole genome sequencing (WGS) by the Human Microbiome Project (HMP) [31]. Rates of HGT among bacteria in the human microbiome (primarily the human GI microbiome) have been estimated to be about 25 times higher than among bacteria in other diverse microecosystems like soil. When evaluating genetic exchange of AMR through HGT between microbes from different ecosystems, the highest rates are observed between microbes from farm animals, human foods and the

human gut [32, 33]. Thus, foodborne and other environmentally-derived AMR elements (e.g., soil) could affect the host directly or indirectly by enhancing the spread of ARG among resident microbial communities through HGT [29, 34]. These scenarios suggest a window of increased susceptibility against AMR in the human microbiome; with gastrointestinal bacteria particularly susceptible due to constant and prolonged environmental exposure [30, 35]. Given recent evidence of the transfer of ARGs in the environment, a more detailed understanding of the dynamics of AMR between the environment and human hosts is merited as the emergence of AMR continues to rise. Likewise, it is necessary to expand AMR mining efforts beyond the GI microbiome. For instance, the oral microbiome also harbors a significant fraction of the human resistome, with genes resistant to tetracycline (e.g. *tet(M)*, *tet(O)*, *tet(Q)* and *tet(W)*), amoxicillin and erythromycin being the most prevalent; flanked by mobile elements such as Tn916 [36–39].

Like the gut, the oral microbiome is also a reservoir of potential ARGs. *Streptococci* seem to be the main carriers of AMR (*tet* genes) in the oral cavity of children, with taxa such as *Veillonella* showing multiple resistance mechanisms (ampicillin, penicillin) [38]. This oral resistome is also observed in the absence of direct selective pressures. Consequently, the challenge ahead relies on characterizing dissemination routes of AMR from the environment into the host and within the host (across body sites). As resistance seems to be ubiquitous in the environment, determining the mechanisms that exacerbate the high prevalence of AMR in animal and human microbiomes, aside from its natural occurrence, is key.

AMR threats and exposure in the early microbiome

As the newborn and infant microbiome are strongly shaped by seeding from the maternal birth canal and maternal skin (mode of delivery) [40, 41], the surrounding primal environment [42, 43] and food stimuli [44–46], understanding early AMR threats in the context of human microbiome maturation and development is critical. For instance, there is evidence that genes resistant to aminoglycoside and β -lactam antibiotics (*BLr*), tetracycline (*Tcr*) and methicillin (*mecA*) are already present in meconium and early fecal samples, in some cases in higher prevalence in newborns compared to mothers (in the case of *mecA*) [47–49]. Furthermore, the presence of AMR elements in the infant gut does not seem to be influenced by selective pressures from foodborne or administered antibiotics [50]. However, in preterm infants, there is evidence of selective negative pressure on meconium bacterial richness depending on antibiotic administered, with ticarcillin-clavulanate, and cefotaxime showing the greatest effects [51, 52]. Thus, as antibiotics are prescribed routinely in preterm and low birthweight infants for preventing necrotizing enterocolitis, spread of AMR in the preterm infant gut should be a factor of concern in the context of long-term intestinal and immune health, and must be addressed in longitudinal studies. Moreover, antibiotic administration during the first 3 years of life, is reported to negatively impact bacterial diversity and enhance abundance of ARGs in the distal gut, with higher persistence of genes flanked by mobile elements [53]. Yet, the long-term health consequences of early antibiotic administration remain unknown.

Consequently, AMR elements may be present in the GI microbiome even before birth, and antibiotic stimuli after birth may further impact the GI microbiome in the first years of life

and primary care stages [54]. The observation that mother-derived AMR bacteria and AMR-containing mobile elements enhance the acquisition and spread of AMR in the infant gut is surprising [55]. For instance, it has been shown that infants and mothers share prevalence of integrase genes (*intI*) containing resistance to sulphonamides, spectinomycin, streptomycin and trimethoprim [56]. *Tcr* genes are also shared between mothers and infants; however, encoded by different organisms; *Bacteroides*, Ruminococcaceae and Clostridiaceae like taxa in mothers; and *Streptococci* in infants [57]. Nonetheless, other reports point to an infant gut resistome that is unique and different from that of the mothers, and that includes resistance to broad-spectrum β -lactam antibiotics, suggesting that early environmental determinants (different from maternal ones) also shape AMR development in the infant gut [58, 59]. In fact, lifestyle factors related to subsistence, diet and other cultural drivers strongly shape the GI microbiome [60, 61].

Dissemination routes of antimicrobial resistance

Antimicrobials and AMR mechanisms in microbial communities are ubiquitous in nature, and have evolved as a natural response of microbes to community dynamics and hierarchical organization [62]. However, AMR in natural microbial communities, including commensal organisms, may be further exacerbated and modified by anthropogenic triggers, constituting a key reservoir for disseminating ARGs. For instance, water [63] and soil [34] host some of the most diverse and widely distributed microbial populations found to carry resistance, thus facilitating genetic exchange and the emergence of novel resistance determinants. In addition, the presence of ARGs in antimicrobial-free habitats [11, 12, 34, 64, 65] and in the stool microbiomes of isolated human populations [66–68] suggest that these mechanisms occur naturally and widely, and that the interconnectedness of close niches contributes to the spread of resistance across different cultures and populations worldwide [69]. For instance, the interconnectedness of soil and farm animals increases the possibility of genetic exchange and selection of novel ARGs [70, 71], with antibiotic resistance then being transferred to human microbiomes through direct contact [72] or by consumption of food-producing animals [73–76]. Indeed, increased animal to human transmission of AMR has been reported since the early seventies, with a focus on antibiotic resistant *Salmonella* [77]. Environmental antibiotic pollution is another likely source of AMR [78, 79]. Antibiotic contaminated hospital wastewater [80] may contribute to the selective pressure and development of bacterial resistance that is then spread to human populations by rodent vectors [81]. Likewise, wastewater treatment plants serving antibiotic production facilities are hotspots for acquiring resistance [82]. With an increase in frequency and ease of travel, resistance quickly disseminates from local to global environments [83–86], further potentiating the establishment of resistance in the commensal gene pool. Studies suggest that 20% to more than 30% of the human gut microbiota may exhibit multidrug resistance in the absence of little to no antibiotic exposure [87, 88], with quinolone resistant alleles existing at notably high frequencies within host-associated bacteria [89]. Analysis of resistance against 68 classes and subclasses of antibiotics in 252 fecal metagenomes of individuals from three countries, Spain, Denmark and the U.S., show that global resistomes are significantly impacted by antibiotics approved for animal use as well as those that have been in use for the longest time [90]. The existence of these global “resistotypes” has also been shown in other studies, pointing to resistance to tetracycline as the most common genotype among

individuals from different nationalities, and increased abundance and diversity of AMR in Spanish, Chinese, French or Italian cohorts compared to cohorts of Americans or Danish people [59, 90, 91].

Of particular importance to human health is the spread of antibiotic resistance in pathogenic bacteria. While resistant variants may emerge within populations of pathogens at the site of infection, commensals may be a more likely source of conferring resistance [92, 93] that is then transferred to pathogenic microbes [94, 95]. Plasmid-mediated quinolone resistance *qnrA* and extended-spectrum β -lactamase resistance *bla*_{CTX-M} determinants originated from nonpathogenic microbes [96, 97]. The largest human-associated population of commensals exists in the gut, thereby increasing their potential of conferring resistance when interacting with transient microbes from the environment harboring ARGs and exposed to antibiotics. Commensal microbes are subject to selective pressure during prophylactic use of antibiotics, and often the dose dependency of antibiotics on targeted tissues is a source of differential selective pressure on commensal populations in nearby niches [98–100]. Close interaction of commensals and pathogens, such as during infection, could provide an opportunity for exchanging resistance mechanisms through HGT.

Detection methods of antimicrobial resistance

High throughput DNA sequencing and bioinformatics have dramatically changed the way AMR is investigated by identifying the genes or mutations that convey resistance. Culture based approaches are used to investigate AMR in the clinical setting using antibiotic sensitivity assays. While this is an effective way to determine if an organism can resist a particular antimicrobial agent, it is dependent on the ability of an organism to be grown in culture, not currently possible for many microbes. The ability to sequence DNA from environmental and clinical samples (metagenomics and whole genome sequencing) to determine gene content presents the opportunity to identify ARGs as well as understand the mechanism of how organisms acquire ARGs. It also presents tools to determine the extent of spread of ARGs amongst diverse populations without the need to first grow the organism in pure culture.

Several sequencing approaches lend themselves to investigating AMR in silico. The ability of an organism to resist a wide range of known antimicrobial agents can be inferred using homology- based methods to predict ARGs. Publicly available databases of ARGs are widely available, with two of the most prevalent being ResFam [101] and CARD [102]. However, the ability to accurately predict AMR phenotype based off homology-based evidence is not without its limitations. For example, many ARGs found in ResFam and CARD are molecular transporters that have been shown to provide AMR by pumping antimicrobial molecules outside the cell, but that resistance may not be present in other organisms that contain a potentially distant homolog of the transporter [103]. This applies to all homology searches that have an intermediate level of homology, where it is not clear if the query gene still possesses the same function as the lab demonstrated ARG [104].

Beyond merely identifying ARGs, lies the task of tracking the spread of AMR from diverse sources to the human microbiome, where they become a serious public health problem. While essential genes are passed vertically when microbial cells divide, potentially useful

genes like ARGs are often gained through HGT [105, 106]. ARGs that all convey a particular resistance can be compared to determine if they were derived from vertical inheritance by one or more groups, or acquired horizontally by taxonomically unrelated groups. When the genome sequence of an organism is available and taxonomy is known, it is possible to determine if an ARG was gained through HGT by comparing the phylogenetic relationship of two distinct organisms, to the homology of their ARGs. Access to complete genome sequences facilitates the ability to distinguish between ARGs located on a plasmid (a common source of horizontally transferred AMR) and ARGs or single nucleotide polymorphisms (SNPs) that are incorporated into an organism's genome. Computational tools such as ACLAME [107] and PhageFinder [108] facilitate detection of mobile elements such as plasmids and prophage which can help to identify the source of a particular ARG detected in a genome or metagenome. Additionally, pangenome analysis using PanOCT allows detection of orthologous ARGs and their presence within genomic islands across multiple related genomes which can help identify ARGs acquired through vertical acquisition or HGT [109, 110]. In the case of metagenomes, where limited gene content is present but taxonomy may not be available, the tetranucleotide frequencies of the DNA sequences can be compared to determine if a portion of the DNA came from a different source [111].

It is evident that AMR has become a global issue, with continuous emergence of new threats [112]. The problem has only become exacerbated with increased international travel, spread of infections in built environments such as hospitals and nursing homes, a global over prescription of antibiotics for both human and animal health, coupled to improper usage when individuals do not follow a completely prescribed course. The concerns associated with emergence of AMR have increased due to a limited understanding of all mechanisms and environmental factors that increase the likelihood of AMR development. In parallel, there has been a major lag in the development of new antimicrobials over the last 15 to 20 years. The majority of our known antibiotics were the result of soil microbial screens, with approximately 3,000 described [113]. Emergence of AMR appears to have superseded the pace at which discoveries and development of better antibiotic treatments are made, and as we move forward, novel approaches to substitute and complement antibiotic therapies are needed [113].

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