

Genomic Insights into the Antibiotic Resistome: A Holistic Approach for Mitigating Antimicrobial Resistance Transmission in One Health

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ABSTRACT

Active investigations into the resistome have been conducted since the inception of the antibiotic resistome concept just over a decade ago. In this study, we revisit the established notion of the resistome, encompassing all forms of antibiotic resistance genes (ARGs), alongside significant findings from each One-Health domain that has embraced this concept. This underscores the pivotal role of the One-Health approach in understanding the dissemination of ARGs. The intricate architecture of the resistome, as observed in the microbiomes of humans, animals, and the environment, necessitates scrutiny through cutting-edge research methodologies. Proposed future directions for resistome research aim to enhance comprehension and management of ARG transmission, building upon recent achievements in resistome studies across various One-Health sectors. These directions include: (1) prioritizing critical ARGs and their associated hosts; (2) elucidating ARG transmission dynamics at the interfaces of One-Health sectors; (3) pinpointing selective pressures influencing the emergence, propagation, and evolution of ARGs; and (4) unraveling the mechanisms facilitating organismal traversal of taxonomic barriers in ARG transmission.

Keywords: Antibiotic Resistance, One Health, Microbiomes, Cutting-edge technologies

Introduction

Antibiotics have been indispensable since the discovery of penicillin, the inaugural antibiotic. However, antimicrobial drug resistance poses a grave concern due to its continual emergence and rapid transmission among pathogens, presenting significant clinical risks. This is unlike other medications used for bacterial infections (1). The introduction of antibiotics into clinical practice has led to the proliferation of resistance, originating from early antibiotics like penicillin and salvarsan, primarily mediated by antibiotic resistance genes (ARGs). In 1973, it was demonstrated that environmental ARGs share genetic similarities with clinical ARGs, indicating actinomycetes, producers of antibiotics, as the source of these clinical genes (2). Subsequently, ARGs were extensively studied only in clinical pathogens for over three decades until the concept of the resistome was introduced. This notion has provided profound insights into the genesis, dissemination, and evolution of ARGs. The objectives of this review are to comprehensively elucidate the genesis, emergence, spread, and evolution of ARGs, as well as to delineate the antibiotic resistome concept and summarize data from the past decade on techniques underlying it. Understanding the intricate structure of the resistome and identifying crucial aspects to mitigate the transmission of ARGs to clinical settings necessitate resistome investigations, particularly from a One-Health (Human, Animal, Environment) perspective (3).

Concept of the antibiotic resistome

The term "antibiotic resistome" was initially coined in 2006 by a team led by Gerry Wright. Initially, they defined it as "resistance determinants present in the soil" and demonstrated that multidrug resistance was more prevalent than previously understood within a population of environmental bacteria (4). Later on, the resistome was defined as a collection of all the ARGs and their precursors in pathogenic and nonpathogenic bacteria. The exact description of its components encompassed all ARGs, including those circulating in pathogenic bacteria, antibiotic producers, and benign nonpathogenic bacteria. Considering the origins of ARGs, it has been suggested that the resistome comprises clinical, environmental, and intrinsic resistance genes, alongside proto-resistance genes, which act as a profound reservoir of ARG precursors as shown in Fig. 1 (3). Subsequent to the publication of numerous studies on the resistome, the definition was refined by categorizing resistance into distinct types: acquired resistance (transmitted either vertically or horizontally, not specific to any taxon), intrinsic resistance (transmitted only vertically, specific to any taxon), silent/cryptic resistance (phenotypically sensitive, functionally present but not expressed), and proto-resistance (phenotypically sensitive, exhibiting little to no activity until mutated) (5). In conclusion, all forms of ARGs (acquired and innate resistance genes), as well as their precursors and potential resistance mechanisms within microbial communities, contribute to resistance through evolutionary processes or alterations in expression context. Since its inception in 2006, the resistome concept has catalyzed significant discoveries regarding antimicrobial resistance (AMR). Important revelations have been proposed and confirmed, including: (i) the long-standing presence of AMR across various microbiomes, (ii) the diverse and intricate nature of the antibiotic resistome, (iii) the environmental resistome serving as both a source and reservoir of

ARGs, (iv) the influence of microbial community structure on the resistome within natural environments, (v) the impact of anthropogenic activities on shaping the environmental resistome, (vi) ARG transmission facilitated by mobile genetic elements, and (vii) the flow of ARGs among humans, animals, and the environment (6). These findings underpin contemporary methodologies, such as the "One-Health" approach, aimed at enhancing our comprehension of AMR within the realms of human, animal, and environmental health (7).

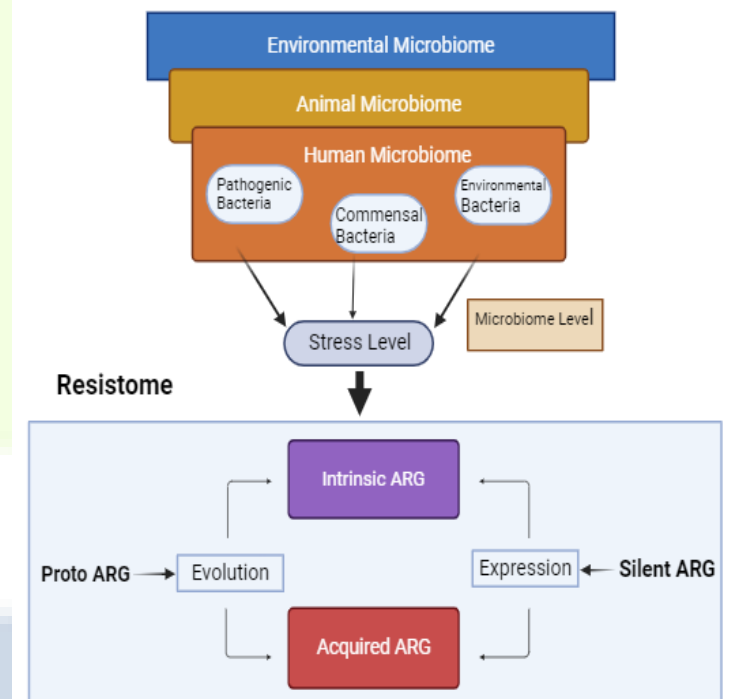


Figure 1: Antibiotic resistome in One-Health sectors

The One-Health concept

The microbiomes of humans, animals, the environment, and various sectors encompassed by the One-Health concept all exchange ARGs, as previously mentioned (8). Described as a collaborative effort of multiple disciplines working locally, nationally, and globally to attain optimal health for people, animals, and the environment through policy, research, education, and practice, One-Health is delineated in academic literature (9). Zoonoses, once recognized as a significant concern for both humans and animals, prompted consultations by the Food and Agriculture Organization (FAO), the World Organization for Animal Health (OIE), and the World Health Organization (WHO) in 2008, emphasizing the relevance of "ecosystems" within the One-Health concept (10).

The primary concerns and objectives of One-Health generally revolve around infectious diseases, particularly zoonoses. At the 2011 tripartite meeting of FAO, OIE, and WHO, antimicrobial resistance (AMR), closely linked to these diseases, was identified as one of the three One-Health priorities(11). A primary challenge associated with the One-Health concept has been recognized as the flow of ARGs among its sectors. While traditionally focusing on relationships and dependencies between various sectors at local levels, the global perspective of One-Health necessitates understanding the interplay between local ecosystems and identifying the factors contributing to the global AMR crisis (12). In the present study, we evaluate the priorities required to mitigate the transmission of ARGs to clinical settings and scrutinize the achievements within each sector.

Environmental resistome

The environmental resistome has undergone comprehensive analysis since introducing the resistome concept in soil environments. ARGs represent widespread and ancient components of bacteria found across diverse ecological settings that harbor various microbial communities(13). The human-made environment can be classified into aquatic and terrestrial settings, while the natural environment can be viewed from a microbial ecology perspective encompassing both. Aquatic environments include freshwater ecosystems such as rivers, lakes, and wetlands, as well as marine ecosystems like seas and estuaries. The terrestrial environment encompasses various terrestrial ecosystems spanning grasslands, forests, deserts, and tundra, across different climate zones. Built environments comprise locations such as wastewater treatment plants (WWTPs), agricultural areas, aquaculture facilities, and hospital settings (14). In this review, our focus is on environmental resistance in agriculture, aquaculture, animals, and humans.

Animal resistome

Antibiotics are utilized in livestock animals for three primary purposes: (a) growth promotion, (b) illness prevention (including prophylaxis and metaphylaxis), and (c) therapeutic veterinary treatment. The latter two reasons exhibit a strong association with animals confined in crowded conditions and the excessive use of antibiotics. Sublethal doses of antibiotics are frequently administered for growth promotion, yet the relationship between these concentrations and weight gain remains unclear(19). Recent research suggests that even minimal antibiotic doses can significantly impact the expansion of an animal's resistome. The use of antibiotics in livestock animals can potentially select for antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARGs), which could subsequently be transmitted to humans (20). Studies on the resistomes of various agricultural animals have indicated positive correlations between antibiotic usage, resistome profiles, and the reliance on mobile genetic elements (MGEs), as evidenced by several regional investigations. A recent study on the resistomes of slaughtered pigs and broilers across nine European countries revealed variations in the quantity and diversity of fecal resistomes based on the respective nation's antibiotic usage(21). Surveys of the resistomes among farm and slaughterhouse workers exposed to animals, conducted in subsequent cross-sectional (human-animal) investigations, revealed the impact of animal resistomes on human resistomes, underscoring the importance of antimicrobial resistance (AMR) surveillance in agricultural animals. Given the complexity of the animal resistome and the diverse environmental variables present at livestock sites, systematic methodologies should be employed for further investigation(22). In addition to livestock animals, wild animals are recognized as sources of ARG spread. The resistome analysis of wild animals in contact with humans displayed a greater diversity of ARGs compared to non-contact animals, suggesting their involvement in transmitting ARGs from animals to humans. Notably, migratory birds among wild animals have been identified as agents spreading ARGs into the environment. Furthermore, the presence of viruses harboring evolving ARGs in companion animals such as dogs and cats suggests their role as reservoirs for antibiotic-resistant bacteria (ARB) and ARGs(23).

Human resistome

The human microbiomes of the respiratory tract, skin, and gut have been extensively investigated using metagenomics based on next-generation sequencing technologies to assess their resistomes(24). Specifically, the transfer of antibiotic resistance genes (ARGs) to pathogenic bacteria, as elucidated by Kim and Cha in *Experimental & Molecular Medicine* (2021), necessitates the regulation of ARG flow from other sectors to the human sector. This underscores the importance of comprehending the dynamics of the human resistome and its interrelationship with other One-Health sectors. The commensal bacterial resistome within the human microbiome is believed to serve as a significant reservoir and conduit for the dissemination of clinical ARGs. Numerous studies have elucidated the role of the human gut resistome through its interpretation and comparison with pathogen resistomes. The presence of nearly identical ARGs and similar genetic contexts between the human gut and pathogenic bacteria underscores the significance of the human gut microbiome in shaping clinical ARGs, despite the rare transmission of ARGs from commensal bacteria to pathogens (25). Established correlations

exist between the environmental and animal gut resistomes and the human gut resistome. Global cohort studies have revealed a national-level resistome structure, with antibiotic usage playing a crucial role in shaping resistome and mobilome structures (a collective term for all mobile genetic element types). The importance of the mobile resistome, which connects ARGs to mobile genetic elements within a genetic framework, has been emphasized in understanding the transfer of ARGs from animals and the environment to pathogens within the complex human gut resistomes. The ongoing discovery of new ARGs in the human gut underscores the gut's role as a reservoir and conduit for ARG transmission (26).

Moreover, numerous studies have been conducted to delineate the dynamics of the human gut resistome during the development of newborns, focusing on the vertical transfer of resistome and mobilome from mother to child. These investigations seek to establish connections between the gut resistome and various illnesses. Evaluation of the human respiratory tract's resistome has been undertaken to elucidate the etiological origins and diagnose infection profiles associated with antimicrobial resistance (AMR) of particular concern is resistance within the respiratory tract microbiome, as individuals with cystic fibrosis and chronic respiratory diseases are prone to polymicrobial infections(27). Additionally, respiratory tract infections significantly impact patient survival in intensive care units. Nonetheless, there is currently a paucity of studies on the respiratory tract's resistome, emphasizing the need for more systematic approaches to characterize the resistome and provide guidance for appropriate antibiotic administration against such infections. The human skin resistomes of hands exposed to the public metro system and uncontacted Amerindian neonates in a neonatal intensive care unit have been described, suggesting a potential role for the skin microbiome in the transmission of antibiotic resistance genes (ARGs)(3).

Future perspectives

The paradigm of antimicrobial resistance (AMR) research has evolved over the past decade, with significant strides made in identifying the origins, transmission, and evolution of AMR. However, since the mechanisms underlying the transmission of antibiotic resistance genes (ARGs) from other One-Health sectors to humans remain only partially understood, future research is expected to build upon the scientific advancements of the previous decade. These endeavors will enhance our understanding of ARG transmission and guide the development of strategies to mitigate the dispersion of AMR(28).

Applications of cutting-edge technologies for antibiotic resistome studies

The antibiotic resistome has been recognized as dynamic and continually expanding, underscoring the importance of cutting-edge technologies to elucidate its dynamics and thoroughly explore its diversity. Advanced sequence-based techniques for resistome research have gained widespread usage across all One-Health domains, owing to the recent development of next-generation sequencing methodologies and bioinformatics tools(29). Metagenomics stands out as a crucial sequence-based method for unraveling the intricate resistome present in microbiomes, with three distinct strategies including targeted gene sequencing, shotgun sequencing, and functional metagenomics. Over the past decade, sequencing, particularly amplicon sequencing, has been extensively employed in numerous investigations, enabling the identification of novel antibiotic resistance genes (ARGs) through functional metagenomics, characterization of ARG variations or genetic contexts via targeted gene sequencing, and resistome-level ARG analysis(30). Various ARG databases, analysis methods, and appropriate sequencing technologies should be tailored to specific research objectives to comprehend antimicrobial resistance (AMR) at the resistome level effectively. The widespread adoption of the SmartChip system, a high-throughput quantitative polymerase chain reaction array, has benefited many environmental microbiomes. This system enables the simultaneous analysis of a large number of ARGs within a short timeframe and offers greater sensitivity than metagenomics for ARG detection. However, it is limited to detecting ARGs and mobile genetic elements (MGEs) that have been previously characterized, precluding the identification of their hosts. To address issues such as missing primers for newly discovered ARGs and redundancy in primer sets, Primer Set 2.0 has recently been released, potentially expanding the applicability of this method for resistome-level analysis despite concerns regarding cost, time, and sensitivity (31). Whole genome sequencing (WGS) for antibiotic susceptibility testing (AST) is increasingly crucial as a potent method for predicting antibiotic susceptibility profiles at the genome level and conducting resistome analysis at the bacterial isolate level. Machine learning and statistical models can aid in this approach, although there is still room for improvement in addressing inconsistencies between WGS and culture-based AST results(32). In summary, these innovative technologies will be pivotal for understanding how bacterial strains or microbiomes transmit ARGs, providing essential insights into the antibiotic resistome within the framework of the One-Health strategy. Perspectives for One-Health-based antibiotic resistance investigations in the future as shown in Fig. 2.

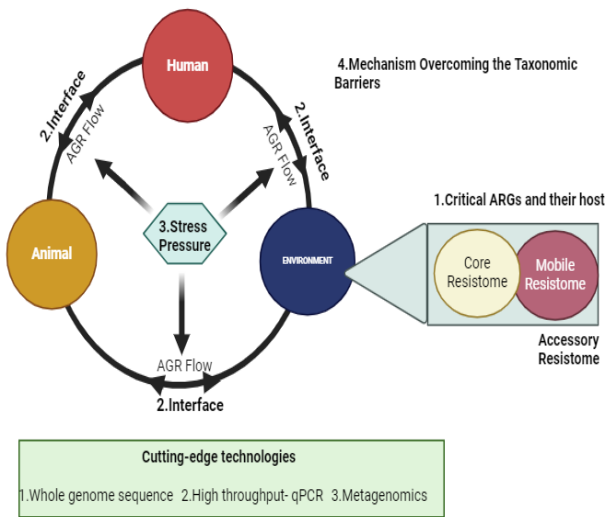


Figure 2: ARG transmission at the interfaces among One-Health sectors

Monitoring the interfaces between these sectors is crucial for understanding the movement of antibiotic resistance genes (ARGs) within the One-Health framework. Therefore, proper configuration of related testbeds and their interfaces is essential(34). By appropriately and concurrently employing culture-dependent and culture-independent approaches, it is possible to obtain snapshots of transmission between the One-Health sectors and comprehend the resistome at these interfaces. The true dynamics of ARG transmission will become evident through the valuable data collected and incorporated via these methods(35).

Selective pressures affecting the emergence, transmission, and evolution of ARGs

Recent research has revealed that anthropogenic activities can alter the environmental resistome; however, precise mechanisms driving changes in the resistomes of One-Health sectors remain poorly understood. It is imperative to gather relevant data meticulously and compare it using reliable statistical methods to discern the selection pressures responsible for these alterations(36). Furthermore, in microcosm or mesocosm studies, monitoring changes in the resistome in response to distinct selective pressures is crucial to obtain empirical evidence. To effectively mitigate antibiotic resistance gene (ARG) transmission to humans, a comprehensive understanding of the relationship between selective pressure and resistome modifications resulting from mitigation efforts is essential(33).

Mechanisms overcoming taxonomic barriers in ARG transmission

Antibiotic-producing bacteria, typically nonpathogenic environmental organisms, have consistently exhibited antimicrobial resistance (AMR). However, at some juncture, due to evolutionary events transcending taxonomic barriers, antibiotic resistance genes (ARGs) became mobile and emerged in clinical settings(37). The precise mechanism underlying the transfer of ARGs from antibiotic producers to pathogens remains unclear, although a case study has proposed a carry-back mechanism. Understanding the mechanisms of taxonomic barrier crossing in ARG transmission is crucial, especially considering the significant proportion of environmental ARGs that remain non-mobile(38).

Conclusion

The concept of the antibiotic resistome and the One Health approach aims to comprehend and diminish the transmission of antibiotic resistance genes (ARGs) across sectors. Despite recent significant findings in antimicrobial resistance (AMR) within these domains, the origins, emergence, dissemination, and evolution of ARGs remain incompletely understood. Several research topics suggested in this study warrant further investigation, facilitated by the utilization of cutting-edge technologies. Such endeavors will contribute to our efforts to effectively combat the ongoing arms race between antibiotics and AMR.

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