
Antibiotic Resistance Genes and Horizontal Gene Transfer in Environmental Microbiomes: A Survey

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Abstract

Antibiotic resistance genes (ARGs) and horizontal gene transfer (HGT) are pivotal in shaping microbial ecology and public health landscapes. This survey paper provides a comprehensive analysis of ARGs and HGT, highlighting their significance in environmental microbiomes. The study begins by elucidating key concepts, such as the role of ARGs in conferring antibiotic resistance and the mechanisms of HGT, including transformation, transduction, and conjugation. It then explores environmental factors influencing ARG dissemination, emphasizing the impact of anthropogenic activities, natural environmental factors, and ecological interactions. The role of mobile genetic elements and biofilms as hotspots for HGT is examined, alongside innovative pathways such as lightning-triggered electroporation. The paper further delves into the ecological and evolutionary implications of ARG dissemination, discussing its effects on microbial community dynamics, structure, and function. Case studies from aquatic, soil, and airborne environments illustrate the real-world implications of ARG spread. The survey concludes with a discussion on public health and environmental management strategies, advocating for integrated approaches to mitigate antibiotic resistance. By synthesizing findings from multiple studies, this paper provides a robust framework for understanding the complex interplay between genetic elements and microbial ecology, emphasizing the need for innovative methodologies to address the challenges posed by ARGs and HGT. Future research directions include expanding genomic data, refining detection methodologies, and exploring therapeutic applications of HGT, with an overarching goal of protecting public health and maintaining ecological balance.

1 Introduction

1.1 Significance of ARGs and HGT

Antibiotic resistance genes (ARGs) and horizontal gene transfer (HGT) are pivotal in microbial ecology and public health. The escalating prevalence of antimicrobial resistance (AMR) substantially threatens public health, leading to increased mortality, extended hospital stays, and rising healthcare costs [1]. The global spread of AMR in bacterial populations is exacerbated by the widespread presence of ARGs across various environments [1]. Notably, the surge in carbapenem-resistant Enterobacterales (CPE) since 2010, driven largely by carbapenemase-producing strains, underscores the urgent need for effective surveillance and control strategies [2].

HGT is a crucial mechanism for genetic exchange, facilitating the rapid dissemination of ARGs and the emergence of antibiotic-resistant bacteria, commonly referred to as 'superbugs'. This genetic exchange is fundamental to prokaryotic evolution, introducing genomic novelty that can lead to the emergence of virulent strains [3]. For example, HGT significantly influences the ecological and genomic plasticity of vibrios, which is essential for pathogen emergence and niche adaptation [4]. Bridging laboratory findings with natural environments is vital for comprehensively understanding HGT dynamics within microbial communities [5].

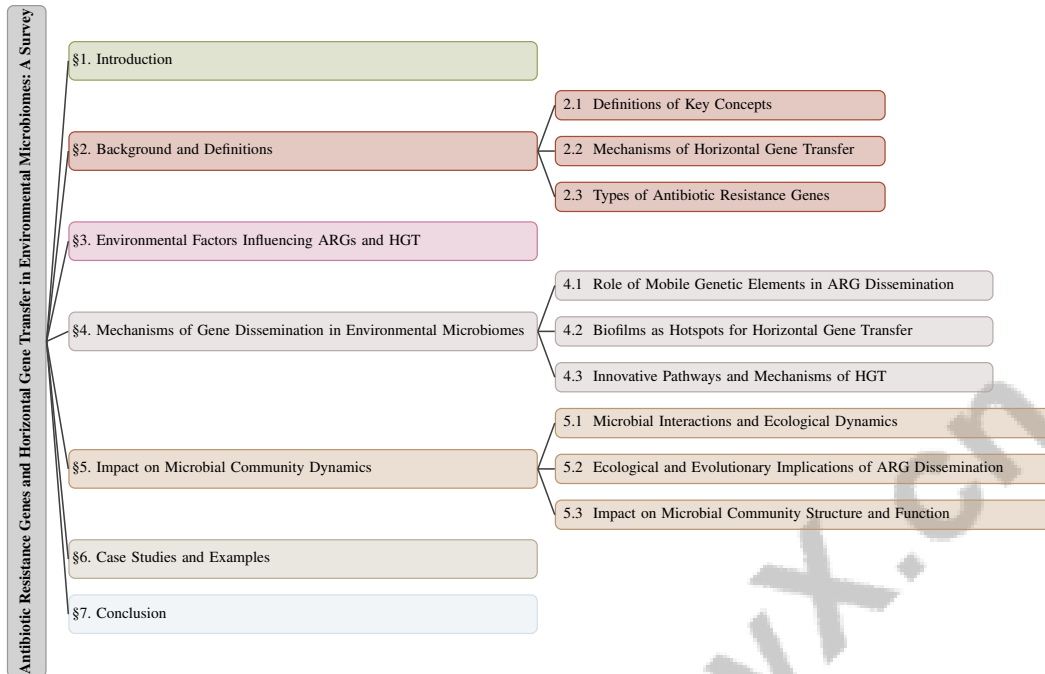


Figure 1: chapter structure

The environmental impact of antibiotics, antibiotic-resistant bacteria (ARB), and ARGs is profound, posing significant challenges for microbial ecology and public health [6]. The prevalence of metallo- β -lactamases (MBL) producing *Enterobacter hormaechei* in France illustrates the public health challenges associated with ARG dissemination [2]. Understanding HGT mechanisms is essential for grasping their implications for genetic diversity, disease progression, and therapeutic challenges [3]. Thus, addressing the ecological and public health ramifications of ARGs and HGT requires an integrated approach encompassing evolutionary biology, microbial ecology, and public health strategies.

1.2 Structure of the Survey

This survey is structured to provide a thorough understanding of the role of antibiotic resistance genes (ARGs) and horizontal gene transfer (HGT) in environmental microbiomes. It begins with an introduction that underscores the significance of ARGs and HGT in microbial ecology and public health, laying the groundwork for subsequent exploration of these topics. The following section clarifies key concepts such as ARGs, HGT, microbial ecology, and environmental microbiomes, establishing a foundational understanding for the subsequent discussions.

The survey then explores environmental factors influencing ARGs and HGT, considering both anthropogenic activities and natural elements that affect the prevalence and spread of these genetic components. It elaborates on the mechanisms of gene dissemination within environmental microbiomes, emphasizing the critical roles of mobile genetic elements (MGEs) and biofilms. Additionally, innovative pathways of HGT are examined, which facilitate the rapid acquisition of diverse genetic traits—including antibiotic resistance and metabolic capabilities—while also shaping the ecological dynamics and evolutionary trajectories of microbial communities. This analysis is further enhanced by advanced computational algorithms and experimental methodologies that elucidate HGT's ecological implications in response to environmental stimuli and selective pressures [7, 5, 8, 9].

Subsequent sections analyze the influence of ARG dissemination on microbial community dynamics, illustrating how these genes modify interactions, shape ecological dynamics, and alter community structure and function. Recent studies highlight the role of HGT in enhancing community diversity and adaptability, alongside the complex interplay between MGEs and microbial populations that can lead to significant evolutionary changes. Moreover, environmental factors such as habitat heterogeneity and landscape dynamics are shown to impact these microbial interactions and the

resilience of communities [7, 10, 5, 11]. Real-world implications are illustrated through case studies focusing on various environmental settings, including aquatic environments, soil and agricultural ecosystems, and airborne ARGs.

The survey concludes with a discussion on the broader implications for public health and environmental management, proposing future research directions to fill existing gaps in understanding ARGs and HGT in environmental microbiomes. Throughout the survey, the integration of findings from multiple studies emphasizes the necessity for new methods to analyze microbial abundance data [12] and the role of HGT in transferring ARGs from food animals to humans [13], thereby providing a robust framework for understanding the intricate interplay between genetic elements and microbial ecology. The following sections are organized as shown in Figure 1.

2 Background and Definitions

2.1 Definitions of Key Concepts

Antibiotic resistance genes (ARGs) are genetic sequences enabling bacteria to resist antibiotics, posing significant public health challenges and threatening microbial ecosystem stability [6]. They are prevalent across various environments, especially in treated wastewaters, where human activities exacerbate their occurrence [6]. The prevalence of ARGs correlates with antibiotic-resistant bacteria (ARB), complicating treatments and raising the risk of multidrug-resistant infections.

Horizontal gene transfer (HGT) is a critical mechanism for non-reproductive genetic material transfer between organisms, significantly influencing microbial evolution and the spread of ARGs [5]. Mobile genetic elements (MGEs) such as plasmids, transposons, and integrons facilitate this exchange between resistant and sensitive bacterial strains [2]. HGT plays a vital role in bacterial genomic evolution, including vibrios, enhancing pathogenicity and ecological adaptability [4].

Microbial ecology examines microorganisms in their natural habitats, focusing on interactions, diversity, and ecosystem roles. It explores microbial community responses to environmental changes, particularly the influence of ARGs and HGT on community dynamics [5]. Environmental microbiomes represent the collective genomes of microorganisms in specific environments, shaped by human activities and natural conditions that alter microbial abundance and community structure [5].

The intricate interrelationships among ARGs, HGT, microbial ecology, and environmental microbiomes significantly impact community dynamics and interactions, often leading to antibiotic-resistant strains that threaten public health and ecosystem stability [6]. The dissemination of resistance genes illustrates the roles of clonal and horizontal transmission mechanisms in shaping microbial communities [2]. Understanding these connections is crucial for developing strategies to mitigate antibiotic resistance and maintain ecological balance, emphasizing the interconnectedness of human, animal, and environmental microbiomes [6].

2.2 Mechanisms of Horizontal Gene Transfer

Horizontal gene transfer (HGT) is a key evolutionary mechanism facilitating rapid genetic material exchange, including ARGs, among microbial communities. This process is primarily mediated through transformation, transduction, and conjugation [3]. Each mechanism uniquely contributes to genetic diversity and adaptability within bacterial populations.

Transformation involves the uptake of free extracellular DNA by competent bacterial cells, allowing them to acquire new traits, including ARGs, enhancing their survival in changing environments [14]. The efficiency of transformation is influenced by environmental conditions and the physiological state of bacteria, determining their competence for DNA uptake [15].

Transduction, mediated by bacteriophages, occurs when bacterial DNA is accidentally packaged into phage particles during viral replication. This DNA, often containing ARGs, is transferred to new bacterial hosts, facilitating genetic exchange and bacterial evolution [3]. Transduction is particularly significant in environments with high bacteriophage activity, such as aquatic systems, enhancing genetic diversity and ARG spread [16].

Conjugation is characterized by the direct transfer of genetic material between bacterial cells through physical contact, typically via plasmids. Plasmids frequently carry multiple ARGs, promoting

the efficient spread of antibiotic resistance across different bacterial species [3]. The efficiency of conjugative transfer is influenced by the compatibility of donor and recipient strains and environmental conditions affecting bacterial motility and contact [15].

Recent research has identified innovative pathways and factors influencing HGT dynamics. The integration of genome-resolved metagenomics with modeling approaches has been emphasized to enhance predictive capabilities in microbial ecology and water engineering, improving our understanding of HGT processes [17]. Additionally, novel methodologies like the LDT graph method have been developed to identify HGT events by defining gene pairs that diverged post-species divergence [18].

The complexity of HGT is further illustrated by frameworks categorizing transfers into innovative and maintenance types, where innovative transfers confer new functions, and maintenance transfers replace lost functions due to endosymbiont degeneration [9]. Understanding these diverse mechanisms and their implications for microbial community dynamics is essential for developing strategies to mitigate ARG spread and maintain microbial ecosystem stability.

2.3 Types of Antibiotic Resistance Genes

Antibiotic resistance genes (ARGs) encompass a diverse range of types, each conferring resistance to various antibiotic classes and contributing to the complexity of antimicrobial resistance across environments. The diversity of ARGs is often shaped by HGT events, facilitating the dissemination of resistance traits within microbial communities [19]. Key types of ARGs include those conferring resistance to β -lactams, tetracyclines, aminoglycosides, and macrolides, frequently associated with mobile genetic elements such as plasmids, transposons, and integrative conjugative elements (ICEs), crucial for their mobilization and spread [8].

In biofilm environments, where high cell densities promote genetic exchange, ARGs are particularly prevalent. Conjugative plasmids and ICEs from Gram-positive bacteria, often underrepresented in reviews, provide significant insight into the types of ARGs found in these structured communities [8]. The presence of ARGs in biofilms underscores their role as reservoirs and hotspots for resistance trait exchange.

The dynamics of ARG dissemination are influenced by interactions between plasmid-carrying resistant cells and plasmid-free susceptible cells, as explored in theoretical models of bacterial population dynamics [15]. These models highlight the role of plasmids in maintaining and spreading resistance within microbial populations.

Moreover, methodologies such as the Synteny Index-based HGT detection (SI-HGT) enhance the detection and analysis of HGT events contributing to ARG diversity. This method utilizes the loss of synteny to statistically assess the likelihood of gene transfer events, providing a robust framework for understanding ARG spread among closely related species [20].

While some studies suggest HGT may influence mutation rates, others argue that data do not support this, emphasizing the need for further research to clarify the relationship between HGT and genetic variation [21]. Understanding the types and mechanisms of ARG dissemination is crucial for developing strategies to combat antibiotic resistance and safeguard public health.

In recent years, the understanding of antibiotic resistance genes (ARGs) and their dissemination has evolved significantly, primarily due to the recognition of various environmental factors that play a crucial role in this process. Figure 2 illustrates the hierarchical structure of these environmental factors, categorizing the impact of anthropogenic activities, natural environmental factors, and ecological influences on gene dissemination. This figure highlights key elements such as human activities, pollution, abiotic and biotic elements, and ecological disturbances, thereby emphasizing their roles in shaping microbial community dynamics and the spread of ARGs. By examining the interactions among these factors, we can better comprehend the complex landscape in which antibiotic resistance emerges and proliferates.

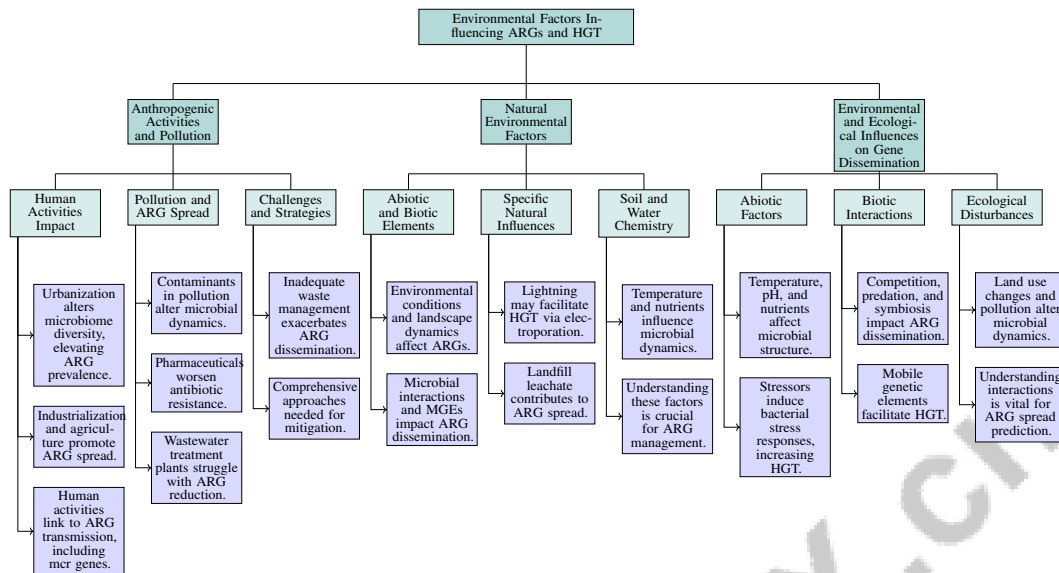


Figure 2: This figure illustrates the hierarchical structure of environmental factors influencing antibiotic resistance genes (ARGs) and horizontal gene transfer (HGT), categorizing the impact of anthropogenic activities, natural environmental factors, and ecological influences on gene dissemination. The diagram highlights key elements such as human activities, pollution, abiotic and biotic elements, and ecological disturbances, emphasizing their roles in shaping microbial community dynamics and ARG spread.

3 Environmental Factors Influencing ARGs and HGT

3.1 Anthropogenic Activities and Pollution

Human activities such as urbanization, industrialization, and agriculture significantly influence the prevalence and spread of antibiotic resistance genes (ARGs) and horizontal gene transfer (HGT) in microbial communities. Urbanization, with its increased population density, alters microbiome diversity, thus elevating ARG prevalence through modified microbial interactions and selective pressures. The transmission of ARGs, particularly *mcr* genes, is intricately linked to human activities, including the food chain, underscoring the substantial impact of anthropogenic factors on antibiotic resistance [2].

Pollution introduces contaminants that alter microbial community dynamics, facilitating ARG spread. Emerging contaminants, like pharmaceuticals, significantly change microbial communities, potentially worsening antibiotic resistance [3]. Wastewater treatment plants, pivotal in ARG dissemination, often struggle to reduce antibiotic-resistant bacteria (ARB) and ARGs despite advanced treatment technologies. The complexity of airborne bacterial communities further complicates ARG control efforts in the atmosphere [2].

In environments impacted by human activities, HGT allows rapid adaptation and genetic diversity among microbial populations. However, the stochastic nature of bacterial reproduction and competition challenges modeling interactions between evolutionary forces and HGT mechanisms [18]. False positives in HGT claims due to contamination or sequence misinterpretation highlight the difficulties human activities pose in accurately assessing ARG prevalence [22].

Inadequate waste management and limited understanding of ARG dissemination exacerbate challenges posed by anthropogenic activities. In agriculture, benchmarking ARGs in soils is crucial for regional comparisons and mitigation strategies for antibiotic resistance pollution [2]. Moreover, environmental factors influence biofilm formation—initial attachment, maturation, and dispersion—key stages for ARG and HGT prevalence and spread.

Addressing these challenges requires a comprehensive approach integrating ecological, evolutionary, and public health perspectives to mitigate the impact of human activities and pollution on ARGs and

HGT. This approach should consider evolutionary innovations facilitated by HGT, enabling organisms to adapt to new environments. Understanding these complex interactions is vital for developing strategies to manage antibiotic resistance spread and maintain ecological balance [3].

3.2 Natural Environmental Factors

Natural environmental factors significantly influence the prevalence of antibiotic resistance genes (ARGs) and the dynamics of horizontal gene transfer (HGT) in microbial communities. Abiotic elements, such as environmental conditions and landscape dynamics, alongside biotic components like microbial interactions and mobile genetic elements (MGEs), collectively impact ARG dissemination, illustrating the complex interplay between microbial community structure and environmental influences, which vary across geographical and seasonal contexts [23, 10, 7, 24].

Lightning strikes may create conditions favorable for electroporation and electrofusion in microbial cells, potentially facilitating HGT by enabling genetic material transfer across cell membranes, thus influencing ARG prevalence in electrically charged environments [25]. This highlights the role of abiotic factors like electrical fields in microbial genetic exchanges.

Landfill leachate contributes significantly to ARG and ARB dissemination in ecosystems. As leachate percolates through landfill sites, it carries diverse contaminants, including ARGs, into groundwater and soil, enhancing environmental reservoirs of resistance genes and complicating microbial resistance management [24]. The interactions between leachate components and microbial communities exemplify the interplay between natural and anthropogenic influences driving ARG prevalence.

Soil and water chemistry, temperature fluctuations, and nutrient availability further illustrate how natural environmental factors affect ARGs and HGT. These factors significantly influence microbial community composition and activity, impacting HGT rates and ARG persistence. Altered microbial dynamics can enhance bacterial adaptability by facilitating the acquisition of novel genetic material, including ARGs, affecting community diversity and pathogenicity. Understanding these relationships is crucial, particularly in contexts like wastewater treatment plants, where mobile genetic elements can yield complex outcomes influencing both microbial ecology and public health [12, 26, 7, 10, 5].

3.3 Environmental and Ecological Influences on Gene Dissemination

Environmental and ecological factors are pivotal in shaping the dissemination of antibiotic resistance genes (ARGs) through horizontal gene transfer (HGT) in microbial communities. These factors create selective pressures and ecological niches that facilitate genetic exchanges, thus maintaining and propagating diverse genetic traits, including ARGs. The study by Cui et al. [11] emphasizes the role of HGT in sustaining diverse defense systems within microbial populations, illustrating how environmental conditions influence gene flow and microbial adaptability.

The complexity of microbial ecosystems is shaped by abiotic factors, such as temperature, pH, and nutrient availability, which affect microbial metabolism and community structure. These conditions can enhance ARG persistence and transfer by selecting for resistant strains and promoting interactions that facilitate HGT. Environmental stressors can induce bacterial stress responses, increasing HGT rates as a survival strategy. This adaptive mechanism allows bacteria to acquire novel DNA, enhancing competition for resources and altering host interactions, including traits like antibiotic resistance. The interplay between environmental stressors and HGT significantly influences bacterial community dynamics and poses implications for human health, particularly regarding antibiotic resistance and pathogenicity. Recent advancements in computational algorithms and metagenomic approaches illuminate the complex ecology of HGT in natural microbial communities, revealing how these processes are shaped by selective pressures and environmental stimuli [23, 7, 5, 4, 27].

Biotic interactions, including competition, predation, and symbiosis, significantly impact ARG dissemination. The presence of diverse microbial taxa can drive genetic material exchange through HGT, as microbes seek advantageous traits for survival. The dynamics of antimicrobial resistance dissemination are shaped by mobile genetic elements, such as plasmids and transposons, which serve as vectors for HGT among diverse microbial populations, including those in livestock and human environments. This gene transfer enhances bacterial adaptability and raises concerns about potential

resistance trait exchange between animal and human pathogens, complicating efforts to manage antimicrobial resistance in food production systems [13, 5, 28].

Ecological disturbances, whether natural or anthropogenic, impact gene dissemination by altering microbial community dynamics and the availability of genetic material for transfer. Disturbances such as land use changes, pollution, and climate change modify habitats and create new opportunities for HGT. A comprehensive understanding of the interactions between environmental factors and microbial ecology is essential for predicting ARG spread and formulating effective strategies to mitigate antibiotic resistance risks across ecosystems. This understanding is critical given the rising prevalence of antibiotic-resistant bacteria (ARB) and ARGs in environments impacted by municipal solid waste, agricultural runoff, and treated wastewater, highlighting the need for innovative management practices to address emerging contaminants and their potential threats to environmental integrity and public health [6, 10, 24].

4 Mechanisms of Gene Dissemination in Environmental Microbiomes

Category	Feature	Method
Innovative Pathways and Mechanisms of HGT	Efficient Detection Methods	SI-HGT[20], LDT-R[29]

Table 1: This table summarizes innovative pathways and mechanisms of horizontal gene transfer (HGT) by listing efficient detection methods. The methods SI-HGT and LDT-R are highlighted for their contributions to identifying HGT events in microbial populations.

Table 2 provides a comprehensive overview of the mechanisms involved in gene dissemination within environmental microbiomes, focusing on the innovative methods used to detect horizontal gene transfer (HGT) and their ecological significance. Understanding gene dissemination dynamics within environmental microbiomes necessitates exploring mechanisms that facilitate genetic material transfer among microbial populations. Central to this exploration is the role of mobile genetic elements (MGEs), which are crucial in the horizontal gene transfer (HGT) of antibiotic resistance genes (ARGs). Table 1 presents a concise summary of innovative horizontal gene transfer (HGT) detection methods, emphasizing their role in understanding gene dissemination within microbial communities. The following subsections detail the contributions of these elements to ARG dissemination, elucidating their mechanisms and ecological implications.

4.1 Role of Mobile Genetic Elements in ARG Dissemination

Mobile genetic elements (MGEs) like plasmids, transposons, and integrons are pivotal in ARG dissemination within microbial communities, enhancing genetic diversity and adaptability through horizontal gene transfer (HGT) [5]. Plasmids, as extrachromosomal DNA capable of autonomous replication, effectively transfer multiple ARGs between hosts, especially under antibiotic selection pressure, thereby promoting bacterial survival [15]. The integration of transformation and conjugation mechanisms in population dynamics models further underscores the complexity of plasmid-mediated gene transfer [15].

Transposons, known as "jumping genes," facilitate ARG spread by moving genetic material within and between genomes, enhancing bacterial adaptability to environmental changes. They promote genetic diversity and evolutionary innovation, with supra-operonic clusters (SOCs) suggested as fundamental HGT units, indicating the involvement of larger genomic units [30].

Integrans capture and express gene cassettes, including ARGs, at a single locus, particularly advantageous in high antibiotic selection environments [6]. The interplay between MGEs and environmental factors drives ARG dissemination, with multi-'omics approaches enhancing understanding of gene dissemination in engineered water systems [17]. This comprehensive approach highlights the complexity of MGE-mediated ARG spread and the need for innovative methodologies to assess their ecological impact.

The role of MGEs in ARG dissemination involves a complex interplay of genetic, ecological, and evolutionary dynamics, necessitating a multifaceted approach to understand and mitigate antibiotic resistance spread [31]. Integrating mechanistic and predictive studies provides valuable insights into ARG spread processes, informing strategies to combat antimicrobial resistance.

4.2 Biofilms as Hotspots for Horizontal Gene Transfer

Biofilms, structured microbial communities adhering to surfaces within a self-produced extracellular matrix, create niches that significantly facilitate HGT and ARG spread. The dense cellular arrangement and proximity within biofilms enhance genetic exchange likelihood, making them critical HGT hotspots [15]. Extracellular polymeric substances (EPS) in biofilms stabilize the community and provide a conducive environment for the accumulation and transfer of MGEs like plasmids, transposons, and integrons [17].

Biofilms' protective nature against environmental stressors, including antibiotics, promotes ARG persistence and dissemination. Bacteria in biofilms exhibit increased antimicrobial resistance due to the EPS barrier and enhanced HGT mechanisms, allowing rapid adaptation under selective pressure [6]. This resistance is often mediated by plasmids carrying multiple ARGs, facilitating resistance trait spread among diverse species [15].

Biofilms also provide a stable environment for bacteriophages, which mediate transduction and facilitate genetic material exchange, contributing to microbial community genetic diversity and adaptability [3]. Phage-mediated transduction, coupled with high cell density and metabolic activity, enhances HGT event frequency and efficiency.

Advancements in multi-'omics approaches have deepened understanding of biofilm interactions driving HGT and ARG dissemination. Integrating metagenomics, metatranscriptomics, and other 'omics technologies offers insights into biofilm-associated community dynamics and MGEs' role in ARG spread [17]. These methodologies provide a comprehensive view of biofilms' genetic and metabolic networks, highlighting their significance as antibiotic resistance reservoirs and conduits.

4.3 Innovative Pathways and Mechanisms of HGT

Exploring innovative HGT pathways and mechanisms reveals complexities beyond classical mechanisms. Lightning-triggered electroporation and electrofusion suggest natural electrical discharges can facilitate HGT by enabling genetic material transfer across microbial membranes [25], highlighting abiotic environmental factors' potential influence on microbial genetic exchanges and ARG spread.

Advancements in computational methodologies have enhanced HGT event detection and analysis. A polynomial-time recognition algorithm for LDT graphs identifies HGT events without explicit tree reconstruction, improving traditional phylogenetic methods [29]. This approach offers a more efficient framework for analyzing complex evolutionary histories and identifying gene transfer events.

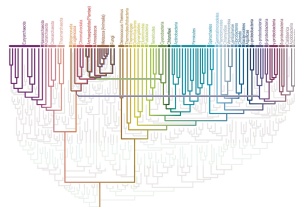
Supra-operonic clusters (SOCs) offer an innovative perspective on gene transfer dynamics, reflecting gene interactions' complexity in bacterial genomes more accurately than traditional operon models [30]. This concept emphasizes considering larger genomic units in gene transfer and bacterial evolution studies.

Adaptive HGT detection approaches, varying criteria based on species distance and gene lengths, have improved HGT event identification specificity [20]. These methodologies allow precise gene transfer event detection across diverse microbial taxa, addressing challenges posed by varying evolutionary distances and genetic complexities.

Recognizing ARG spread control complexity has led to identifying new therapeutic targets to limit HGT and combat multidrug resistance [14]. This emphasizes novel strategies and interventions to address antibiotic resistance challenges.

These innovations highlight HGT's dynamic nature and role in microbial evolution and ARG dissemination. Leveraging advanced computational techniques and considering environmental factors' complex interplay enhances understanding of HGT mechanisms in microbial communities. This insight elucidates genetic exchanges' role in antibiotic resistance spread and informs targeted strategies to mitigate this public health threat. Studies reveal MGEs' diverse roles in facilitating HGT and their impact on microbial population dynamics, underscoring effective interventions' need to consider ecological context and evolutionary pressures [7, 13, 5, 1].

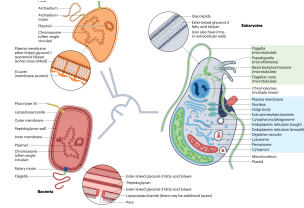
As shown in Figure 3, the mechanisms of gene dissemination in environmental microbiomes are a fascinating area of study, particularly when exploring innovative pathways and mechanisms of horizontal gene transfer (HGT). This example introduces visual representations encapsulating life's diversity and the intricate processes involved in genetic transmission across organisms. "The Tree of



(a) The Tree of Life: A Visual Representation of Life's Diversity[25]

	In vitro quantification	In vivo quantification	In situ & metatranscriptomic quantification
Definition	Quantifying HGT rates under highly controlled bench-top laboratory conditions	Measuring HGT occurrence in representative environmental communities	Observing and inferring naturally occurring HGT
Advantages	<ul style="list-style-type: none"> Clear quantification of HGT rates Controlled environment allows for mechanistic insights Ability to study specific HGT events 	<ul style="list-style-type: none"> Relevant to natural ecological processes Captures higher-order interactions Measures HGT in complex communities 	<ul style="list-style-type: none"> Direct evidence of natural HGT Captures HGT dynamics in real-world environments Provides insights into the prevalence of HGT in nature
Disadvantages	<ul style="list-style-type: none"> Simplified environmental conditions may not reflect natural complexity Artificially high or low HGT rates Lack of ecological context 	<ul style="list-style-type: none"> Difficult to control over experimental conditions Measurement noise and variability Requires sophisticated data analysis 	<ul style="list-style-type: none"> Methodological challenges (e.g., low signal-to-noise ratio) Requires specialized equipment and expertise HGT is inferred and not directly observed

(b) Quantification of Human Genetic Transmission (HGT) Rates Across Different Scales: A Comparative Analysis[7]



(c) The image compares the structures of bacteria and eukaryotes.[32]

Figure 3: Examples of Innovative Pathways and Mechanisms of HGT

"Life" provides a colorful depiction of evolutionary relationships among various life forms, highlighting interconnectedness and diversity within the biosphere. The second image offers a comparative analysis of human genetic transmission rates, illustrating differences in quantification methods such as "In vitro," "In vivo," and "In situ metatranscriptomic," emphasizing HGT's significance in understanding genetic flow and adaptation. The third image delves into structural distinctions between bacteria and eukaryotes, providing insight into cellular features influencing gene transfer mechanisms. Together, these visual examples illuminate complex pathways through which genes are disseminated in environmental microbiomes, showcasing innovative mechanisms facilitating HGT and contributing to life's dynamic evolution [25, 7, 32].

Feature	Role of Mobile Genetic Elements in ARG Dissemination	Biofilms as Hotspots for Horizontal Gene Transfer	Innovative Pathways and Mechanisms of HGT
Transfer Mechanism	Plasmid-mediated Transfer	Enhanced Genetic Exchange	Electroporation And Electrofusion
Environmental Influence	Antibiotic Selection Pressure	Biofilm Protective Nature	Abiotic Electrical Discharges
Detection Methodology	Population Dynamics Models	Multi-omics Approaches	Computational Methodologies

Table 2: This table provides a comparative analysis of three key aspects of horizontal gene transfer (HGT) in environmental microbiomes: the role of mobile genetic elements in antibiotic resistance gene dissemination, biofilms as hotspots for genetic exchange, and innovative pathways and mechanisms of HGT. The table highlights differences in transfer mechanisms, environmental influences, and detection methodologies, offering insights into the complexity of HGT processes.

5 Impact on Microbial Community Dynamics

5.1 Microbial Interactions and Ecological Dynamics

Antibiotic resistance genes (ARGs) significantly reshape microbial interactions and ecological dynamics by altering genetic landscapes and evolutionary pathways. Horizontal gene transfer (HGT) enhances genetic diversity and adaptability, enabling bacteria to withstand environmental and anthropogenic pressures [8]. This genetic exchange is crucial for microbial evolution and the persistence of antibiotic resistance across ecosystems.

Advanced methodologies have quantified HGT rates, providing insights into microbial evolution and resistance mechanisms [7]. *Vibrios* exemplify ecologically significant bacteria, playing vital roles in biogeochemical processes and as pathogens, highlighting HGT's impact on community dynamics [4]. The presence of ARGs in environments, including foodborne pathogens, poses public health risks, as these genes can transfer from environmental reservoirs to human pathogens [33].

Biofilms are critical hotspots for ARG dissemination, where dense microbial communities promote HGT and resistance trait persistence [8]. The protective biofilm environment enhances antibiotic-resistant bacteria (ARB) survival and trait coexistence, as stochastic adaptive dynamics models demonstrate dormancy and HGT as key evolutionary forces [34]. In engineered water systems, microbial interactions and functions related to antibiotic resistance are shaped by environmental conditions, with multi-omics approaches offering comprehensive insights into genetic and metabolic networks [17].

Systematic analyses of microbial genomes reveal frequent shared sequences across genera, indicating prevalent HGT and ARG dissemination beyond species boundaries [35]. This exchange enhances microbial resilience and adaptability in diverse environments. In settings like Dutch hospitals, clonal dissemination is the primary mode of vanA-type resistance spread, with HGT playing a role in specific outbreaks [36].

The multifaceted impact of ARGs on microbial interactions and ecological dynamics necessitates a thorough understanding of ARG dissemination mechanisms. Developing innovative strategies for monitoring and controlling antibiotic resistance in microbial ecosystems, particularly in agriculture and wastewater-impacted environments, is essential. Such understanding enables effective remediation methods, like constructed wetlands, to reduce ARB and ARG prevalence [6, 37, 24].

5.2 Ecological and Evolutionary Implications of ARG Dissemination

The horizontal gene transfer (HGT) of antibiotic resistance genes (ARGs) has complex ecological and evolutionary implications for microbial communities. HGT shapes microbial genetic diversity, enabling rapid adaptation to environmental and anthropogenic pressures. While infrequent in vertebrate genomes [32], HGT is prevalent in microbial ecosystems, facilitating ARG spread and impacting microbial evolution.

Mutation rates and genetic diversity are influenced by factors beyond HGT [21], with HGT serving as a double-edged sword—driving genetic innovation while challenging human health, particularly concerning antibiotic resistance [3]. Methodological advancements, such as the SI-HGT method, enhance HGT event detection specificity, especially among closely related species [20], deepening our understanding of ARG dissemination dynamics.

Challenges remain in fully understanding non-canonical HGT methods and environmental influences [14]. Host immune system roles in managing antimicrobial resistance further complicate ARG dissemination's ecological implications [1]. Competitive interactions between sensitive and resistant populations, driven by growth and gene transfer rates, exemplify ARG spread's ecological dynamics [15]. Supra-operonic clusters (SOCs) offer a nuanced understanding of gene transfer patterns beyond traditional operon models.

5.3 Impact on Microbial Community Structure and Function

The horizontal gene transfer (HGT) of antibiotic resistance genes (ARGs) profoundly influences microbial community structure and function. ARGs introduce new genetic traits, modifying interactions, community composition, and ecosystem functions [6]. ARG integration into microbial genomes shifts community dynamics, granting resistant strains a selective advantage and influencing microbial diversity and stability [15].

ARG presence alters microbial communities' functional capabilities, enabling antibiotic degradation and modifying nutrient cycling and energy flow. This shift is pronounced in high antibiotic selection pressure environments, like wastewater treatment plants, where communities adapt to resist and degrade antibiotics [17]. ARG integration enhances metabolic versatility, allowing communities to exploit new niches and resist stressors [3].

ARG spread can alter microbial taxa's ecological roles, affecting host and competitor interactions, potentially increasing competition or cooperation among species, further influencing community structure and function [8]. Mobile genetic elements, like plasmids and integrons, facilitate rapid resistance trait dissemination across populations [5].

Understanding ARGs' complex influence on microbial community structure and function is crucial for managing antibiotic resistance and maintaining ecosystem health. Multi-'omics approaches and computational models provide insights into ARG dissemination mechanisms and effects on microbial ecosystems [17], helping mitigate antibiotic resistance spread and preserve ecosystem functions.

6 Case Studies and Examples

6.1 ARGs and HGT in Aquatic Environments

Aquatic environments serve as significant reservoirs for antibiotic resistance genes (ARGs) and horizontal gene transfer (HGT), influenced by emerging contaminants (ECs) and human activities. While the impact of antibiotics in these ecosystems is well-documented, the effects of non-antibiotic ECs on microbial communities remain underexplored, highlighting a critical gap in understanding their ecological impacts [26]. A metagenomic analysis of 4572 samples identified 2561 ARGs, emphasizing the health risks associated with antibiotic resistance in aquatic systems [27]. The global prevalence of *mcr* genes in 22

Studies on antibiotic-resistant bacteria (ARB) and ARGs in landfill leachate reveal how anthropogenic pollution interacts with microbial communities, enhancing ARG dissemination through HGT [24]. Constructed wetlands (CWs) have shown superior efficacy over traditional wastewater treatment in removing antibiotics and ARGs, offering a sustainable solution to mitigate antibiotic resistance in aquatic environments [6]. The presence of ARGs in treated wastewater and the potential of CWs to reduce their impact highlight the urgent need for ongoing monitoring and management strategies to address the growing challenges of antibiotic resistance [6, 37, 24]. Understanding ARGs and HGT dynamics in these ecosystems is crucial for developing effective interventions to protect public health and ecological balance.

6.2 Soil and Agricultural Ecosystems

Soil and agricultural ecosystems are vital for understanding ARGs and HGT, heavily influenced by agricultural practices, including antibiotic and fertilizer applications. Soil acts as a reservoir for intrinsic antimicrobial resistance, with human activities creating sustained selection pressures that promote ARG prevalence. In the Yangtze River Delta, multiple ARG classes, notably *sul II* and *tet G*, were identified in agricultural soils, with HGT facilitating gene spread among bacteria, raising concerns about transmission pathways from livestock to humans [37, 13]. Manure and organic amendments introduce antibiotics and resistant bacteria, creating selective pressure that enhances ARG persistence and dissemination through transformation, transduction, and conjugation.

Quantitative analyses reveal widespread resistance genes, including those conferring resistance to sulfonamides, tetracyclines, and quinolones in agricultural soils [37]. These findings highlight the interplay between soil properties, chemical contaminants, and microbial communities, necessitating comprehensive monitoring and management strategies to mitigate antibiotic resistance. Mobile genetic elements, such as plasmids, further complicate soil's role as an ARG reservoir, facilitating horizontal transfer among soil bacteria. Future research should focus on contamination source identification and plasmid characterization to enhance understanding of ARG dissemination mechanisms in soil ecosystems [2].

Comparative studies, like those examining vancomycin-resistant *E. faecium* isolates from Dutch hospitals, provide insights into resistance dissemination modes in clinical and environmental contexts [36]. These studies underscore the importance of integrated approaches that consider both clinical and environmental data to understand ARG dynamics in soil and agricultural ecosystems. The significant levels of ARGs in agricultural soils, influenced by human activities and emerging contaminants, highlight the need for focused research to develop effective strategies to combat antibiotic resistance and protect public health [37, 6, 24].

6.3 Airborne ARGs and Seasonal Variations

Airborne ARGs pose a significant public health risk due to their long-distance transport, facilitating widespread resistance trait dissemination. Airborne transmission is a major exposure pathway, particularly through inhalation, with seasonal variations in ARG abundance observed across different regions in China. Geographical patterns show specific ARG profiles consistently associated with certain bacterial genera, and the correlation between ARGs and mobile genetic elements suggests a complex interplay enhancing environmental persistence and potential human exposure [23, 37, 24, 27].

Seasonal changes significantly influence airborne ARG concentrations, with warmer months showing increased microbial activity and bioaerosol presence contributing to ARG dissemination. Regional studies indicate greater seasonal variations in temperate urban areas compared to subtropical regions, while colder temperatures reduce microbial viability and ARG prevalence [23, 10, 24, 27]. Human activities, such as agriculture and industrial emissions, introduce resistant bacteria and genetic material into the atmosphere.

The complexity of airborne ARG dissemination is compounded by microbial community interactions and environmental factors. Biofilms on surfaces may serve as ARG reservoirs, released into the air through disturbances. Additionally, natural phenomena like lightning may facilitate HGT through electroporation, emphasizing abiotic factors' role in ARG spread [25].

To address airborne ARG challenges, comprehensive monitoring and management strategies are essential, considering seasonal variations and environmental influences on ARG dissemination. Advanced methodologies like metagenomics and bioinformatics can elucidate the sources and dynamics of airborne ARGs, particularly in regions like China, where significant seasonal and geographical variations have been observed. This understanding is crucial for developing targeted strategies to mitigate the public health and environmental impacts of inhalational ARG exposure, especially in urban areas with high fine particulate matter (PM 2.5) levels [23, 12].

6.4 Comparative Studies and Methodological Approaches

Comparative studies in ARG and HGT research are crucial for elucidating methodologies used to explore these processes, especially regarding HGT's role in microbial community adaptation and antibiotic resistance emergence. Recent advancements in computational algorithms and experimental techniques have enhanced HGT event detection and their ecological implications, shedding light on gene transfer dynamics in environments like the human gut microbiome [5, 20]. These studies emphasize HGT's effectiveness in disseminating ARGs across microbial communities, highlighting the importance of methodological rigor in evaluating HGT processes.

Benchmarks in HGT research enhance understanding of gene transfer events and provide frameworks for assessing ARG dissemination's impact on microbial evolution. These frameworks enable systematic comparisons across diverse studies, enriching understanding of microbial community dynamics and assembly factors [10, 12]. Comparative analysis of microbiome interactions underscores methodological variations that can inform ARG and HGT research, highlighting the need for standardized approaches to accurately capture microbial interaction dynamics.

Innovative methodologies, such as the LDT graph method, demonstrate the potential for novel techniques to identify a significant proportion of HGT events with high accuracy, providing a robust framework for future evolutionary biology research [18]. The integration of multi-omics methodologies, including genome-resolved metagenomics and whole community gene expression surveys, enhances understanding of microbial communities by offering mechanistic insights into their functions and interactions, facilitating predictive frameworks for sustainable bioprocesses in fields like water engineering [17, 31, 12].

Future research should prioritize expanding genomic data across diverse lineages and refining methodologies to better detect and analyze HGT, particularly in underrepresented groups. This expansion will enhance understanding of HGT's evolutionary implications and its role in shaping microbial diversity and function. Comparative studies and methodological advancements in ARG and HGT research are vital for assessing health risks posed by ARGs, particularly those linked to multidrug resistance, and for developing strategies to mitigate resistance transmission from agricultural environments to human populations. Employing metagenomic analyses and machine learning techniques can map and quantify ARG risks across habitats, informing public health initiatives aimed at controlling antibiotic resistance spread [13, 27].

7 Conclusion

7.1 Implications for Public Health and Environmental Management

The pervasive spread of antibiotic resistance genes (ARGs) and the facilitation of horizontal gene transfer (HGT) present formidable challenges to public health and environmental management,

necessitating comprehensive strategies to address these issues. The rapid proliferation of ARGs, particularly within biofilms, complicates infection treatment and intensifies the global antibiotic resistance crisis. This scenario underscores the necessity for integrated genomic and epidemiological approaches to effectively counteract this growing threat. The detection of *mcr* genes in environmental samples highlights the urgency for effective control measures to mitigate antibiotic resistance risks. These observations emphasize the critical need for global collaboration in monitoring and managing antibiotic resistance, given the widespread presence of ARGs and their profound implications for public health and environmental policy.

Constructed wetlands emerge as a promising intervention for reducing antibiotics and ARGs in treated wastewater, showcasing their potential as an alternative wastewater treatment solution and underscoring their significance in environmental management. The application of multi-omics approaches in microbial research provides valuable insights into the genetic and metabolic networks that drive ARG dissemination, highlighting the importance of interdisciplinary research in addressing the complexities of antibiotic resistance. This integration is particularly relevant for enhancing public health and environmental management, especially within water engineering frameworks.

Future research should focus on elucidating the mechanisms of HGT, developing strategies to mitigate its negative impacts, and exploring its potential therapeutic applications. Additionally, it is crucial to investigate the dynamics of diverse bacterial species and devise tailored strategies for specific regions and bacterial populations to effectively tackle the challenges posed by ARGs and HGT. Further exploration into extending algorithms to unrooted trees and higher-degree trees, as well as improving approximation ratios, is also warranted.

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