Review

Bacteriophages: Potential Candidates for Dissemination of Antibiotic Resistance Genes in the Environment

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Review

Bacteriophages: Potential Candidates for Dissemination of Antibiotic Resistance Genes in the Environment

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Abstract: The discovery of antibacterial drugs i.e., antibiotics marked a monumental achievement in the history of mankind, and this discovery revolutionized how infectious diseases were treated, saving countless lives. Lately, antibiotic resistance has emerged due to the overuse of antibiotics, which in turn poses a significant global threat. Antibiotic resistance genes (ARGs) are disseminated among various biomes and bacterial taxa by lateral or horizontal gene transfer (HGT), involving conjugation, transformation, and transduction mechanisms. This review focuses on transduction, wherein bacteriophages or phages mediate gene transfer between bacteria. Bacteriophages, as prevalent as their bacterial counterparts and often surpassing them in abundance, exert significant control over bacterial populations. According to estimates, 25% of the total genes of *Escherichia coli* originate from other bacterial species because of the HGT mechanism. Transduction can occur through generalized or specialized mechanisms, facilitated by the ubiquitous presence of phages in nature. Metagenomic studies of phages and virus-like particles (VLPs) have revealed the appearance of ARGs and mobile genetic elements (MGEs) of bacterial origin. These genes confer resistance to antibiotics when transferred to bacteria via transduction. Through phage-mediated transduction, ARGs are disseminated from the environment to bacteria associated with humans or animals, underscoring the importance of understanding and addressing this mechanism in the context of antimicrobial resistance.

Keywords: antibiotic resistance genes; bacteriophages; transduction; environment

1. Introduction

Antibiotic compounds, originating from a plethora of microorganisms, have long been in existence before their therapeutic potential in combating bacterial diseases was fully realized by humans. These antibiotics encompass various types tailored to address infections caused by bacteria, fungi, and other microorganisms. The discovery of antibiotics hinges upon understanding their chemical structures and activity spectra [1]. Antibiotics are classified based on the scope of their action, falling into narrow, broad, or extended categories. Narrow-spectrum agents primarily target gram-positive bacteria. Whereas, broad-spectrum antibiotics combat gram-negative and gram-positive bacteria alike, however, extended-spectrum antibiotics are modified chemically and exert influence over additional bacterial types, typically favoring gram-negative strains [2]. Each antibiotic manifests distinct mechanisms of action, e.g., the retardation of protein, cell wall, and nucleic acid formation in bacterial cells. The most prevalent antibiotic classes include penicillins, macrolides, cephalosporins, and fluoroquinolones [3].

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The problem of antimicrobial resistance has emerged as an important event with the widespread use of antibiotics in agriculture and clinical settings, as it poses a substantial threat to public hygiene in the 21st century. According to the latest Lancet report, antimicrobial resistance in 2019 contributed to approximately 4.95 million deaths, with 1.27 million deaths directly linked to this resistance [4]. Without effective control measures, it is projected that this figure could rise to as high as 10 million by the year 2050. Recent studies highlighted certain environmental sources containing clinically resistant pathogens with Antibiotic Resistant Genes (ARGs) [5]. While numerous genes can impart resistance, assessing the comparative health risks associated with ARGs proves complicated. Factors like abundance, potential for lateral transmission, and the capacity of ARGs to be activated in infectious agents all contribute significantly [6]. Consequently, ARGs are increasingly recognized as a new form of environmental pollutant and have garnered significant attention as a global research focus [7]. Antibiotics as a global health concern were declared by many health organizations in the world such as Center for Disease Control (CDC) and the World Health Organization (WHO) [8].

WHO has declared that the rising antimicrobial resistance among bacterial species presents a global concern, representing a significant public health issue. The global concern regarding antibiotic resistance needs more attention from scientists [9]. Antibiotic resistance in bacteria is a natural process. In addition to mutation in several genes residing on bacterial chromosomes, there are genetic exchange mechanisms between microorganisms that constitute an important role in antimicrobial resistance. One of the most important genetic materials is a plasmid which has antibiotic resistance genes. Transmission of these resistance elements is induced by antibiotics and the selective pressure due to these antimicrobial substances is the primary reason for resistance [10,11].

The advent of antibacterial resistance has gained more interest in the form of phage therapy, in which phages can be used as an option for replacement in antibiotic resistance cases. On the other hand, phages play a core role in antibiotic resistance genes dissemination in our environment for all kinds of organisms including human beings. In the current review, we emphasize the ARG's dissemination through bacteriophages in our universe.

1.1. Bacteriophages: Abundance in the Environment

Bacteriophages, as prevalent as their bacterial counterparts and often surpassing them in abundance, exert significant control over bacterial populations. They achieve this through mechanisms such as lysis, leading to the transformation of bacterial immunity systems, facilitating lateral gene transfer, and modulating the metabolic system of the host via the transfer of supplementary metabolic genes. At their core, bacteriophages are essentially nucleic acids encapsulated within a protein capsid. Despite their simplistic structure, these tiny biological entities exert considerable influence in the microbial realm, serving as expert manipulators. The abundant presence of phages underscores their pivotal role in regulating bacteria and shaping the ecology of various environments through the lysis of bacterial hosts, just as they can influence the cycling of organic matter on a large scale by releasing organic material through bacterial cell lysis alongside influencing microbial diversity by selecting for microorganisms resistant to their attacks, thereby altering the proportions of bacterial strains within communities [12].

Bacteriophages are abundantly distributed throughout various habitats on Earth. With an estimated 10^{31} phage particles globally, they outnumber bacterial populations by a factor of ten, making them the biological entities having the most prevalence in the biosphere [13]. In the human body, which hosts over 10^{12} bacteria, particularly in the gut, phages are also ubiquitous, surpassing bacterial numbers by at least tenfold [14]. They play crucial roles in shaping bacterial communities across different bodily sites, like the urinary tract, respiratory tract, gastrointestinal tract, and oral cavity [15]. In ocean environments, studies indicate that phages reign as rich biological entities, with an estimated 4×10^{30} viruses present, indicating that viruses outnumber bacteria and archaea by 15-fold [16]. Similarly, in various soil types worldwide, phage densities range around 10^{10} per gram of dry soil, with minimal variance across different soil types [17]. However, the virus-to-bacterium (VBR) ratio varies to a great extent among soil types, with the counts of viruses being 10- to 100-fold

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lesser than bacteria in soils of agricultural and desert lands, but in Antarctic soil, the counts of viruses are 1000-fold than bacteria [13]. Due to the ample and persistent nature of phages in our environment, the phages help to disseminate the genes responsible for antibiotic resistance among the bacterial cells even in different biome or taxon groups as shown in (Figure 1).

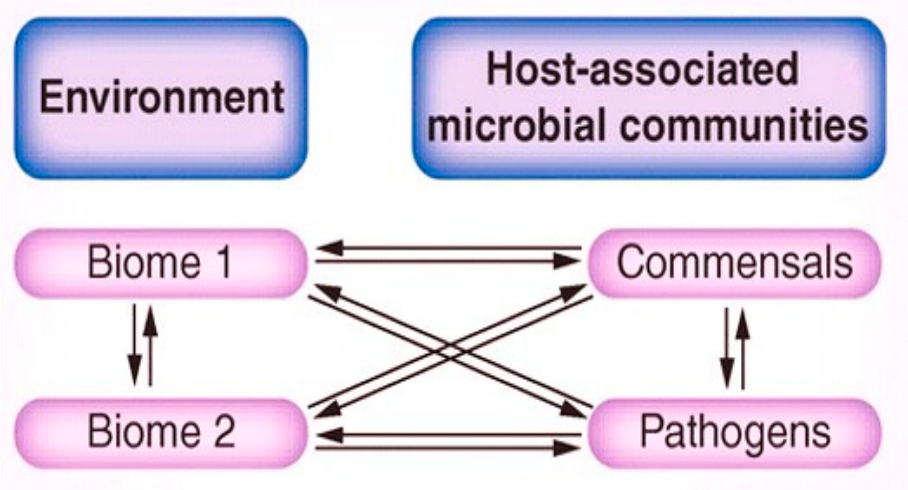

Figure 1. Transfer of antibiotic resistance genes occurs among various ecosystems and microbial communities.

1.2. Bacteriophages: A Vehicle for Resistance Genes

The viruses that infect and replicate specifically within bacterial cells are called phages or bacteriophages. They vary widely in size, morphology, and genomic structure, but all possess a DNA/RNA genome, encapsulated in a phage-encoded protein coat called capsid. Despite diverse appearances, phages are non-motile and rely on Brownian motion for movement [18]. While resistance against antibiotics by microbes is innate, the extensive utilization of antimicrobials has extensively led to this mechanism's prevalence among disease-causing bacteria in animals and humans alike. Many pathogenic species related to human health harbor resistance genes within their chromosomes as an integral component. Various studies indicate that the mechanisms underlying antibiotic resistance observed in clinical settings closely mirror those found in environmental contexts. The extensive mingling of bacteria residing in the environment with bacteria arising from sources related to humans creates ecological conditions that lay the foundation for the advent of antibiotic-resistant strains [19,20]. ARGs can be obtained and disseminated among bacteria via mobile genetic elements, like conjugative plasmids, insertion sequences, integrons, transposons, and bacteriophages [21]. Our review underscores the notable function of bacteriophages in facilitating the transfer of resistance gene elements from environmental reservoirs to pathogens associated with human health, rendering antibiotics ineffective. To mitigate the health issues related to common people associated with antibiotic resistance, it is important to understand the sources and procedures behind the emergence of antimicrobial resistance.

1.3. Antibiotic Resistance Genes Transmission Mechanisms

ARGs can transfer between or among bacterial strains through vertical and horizontal gene transfer mechanisms [22]. The transfer of genes is done through horizontal gene transfer (HGT), including ARGs, from one bacterial strain to another, across different bacterial species or within the same species. In addition to ARGs, other genetic elements such as those encoding virulence factors and metabolic traits can also be transferred through HGT. According to estimates, 25% of the total genes of *Escherichia coli* originate from other bacterial species as a result of the HGT mechanism [23]. Lateral gene transfer mechanisms primarily encompass transformation, transduction, and conjugation [24].

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1.3.1. Conjugation

The process of conjugation occurs by the transferring of genetic material either through plasmid DNA or direct cell-to-cell contact from one bacterium to the other. This process is dependent on the exchange of MGEs like plasmids and integrating and conjugation elements (ICEs) by a pore or pilus formation between closely situated bacterial strains [25]. The transfer of ARGs through plasmid-mediated conjugation poses a serious risk to the health of humans because of the transmission of drug resistance. Research has shown that mechanisms for transmitting drug resistance via ICEs may be observed in Gram-positive bacteria, like *Streptococcus* species [26].

1.3.2. Transformation

Transformation is the process by which recipient bacteria absorb external DNA, primarily plasmid DNA or fragmented DNA produced during bacterial lysis or active secretion [27]. This acquired DNA is then integrated into the genomes of the recipient bacteria, enabling them to acquire new traits [28]. Studies have demonstrated that under natural conditions, *Escherichia coli* can transform by absorbing plasmid DNA, suggesting that *E. coli* can absorb DNA in the digestive tract. It is acknowledged that one possible mechanism influencing the spread of genes resistant to antibiotics is transformation [21].

1.3.3. Transduction

To facilitate the acquisition of new features, transduction uses bacteriophages to function as carriers to transmit chromosomal and extrachromosomal DNA from donor bacteria to recipient bacteria. Phages can accompany antibiotic-resistance genes (ARGs) within the same environmental niche and bacterial populations, implicitly implying their potential part in the dissemination of genes resistant to drugs[29]. Methicillin-resistant strains of *Staphylococcus aureus* are more prone to resistance transduction, whereby they obtain the *mecA* gene from other bacterial species by phage-mediated transduction. The occurrence of transduction in nature is unpredictable, highlighting its profound significance in the transmission of drug resistance, extending beyond conventional understanding [30].

2. Resistance Genes Transmission Mechanisms in Bacteriophages

Bacteriophages facilitate genetic exchange through generalized and specialized transduction, enabling generic material transfer from donor to receiving cells (Figure 2). They exhibit high host specificity and typically infect only a single bacterial species or specific strains. Upon latching onto a host, phages adapt to go through either a lytic or lysogenic cycle of replication. The lytic cycle is characterized by the injection of viral genome in the host cell by the phage, this genome later on hijacks the ribosomes of the host to produce viral proteins. This then leads to the rapid synthesis of new phages and this later on leads to the eventual lysis of the host cell, releasing progeny phages to infect other cells. The lysogenic cycle is characterized by the integration of the phage genome into the bacterial chromosome which replicates alongside the host genome without causing immediate cell death. These integrated phage genomes, known as prophages, can revert to the lytic cycle under certain conditions, leading to host cell lysis [31].

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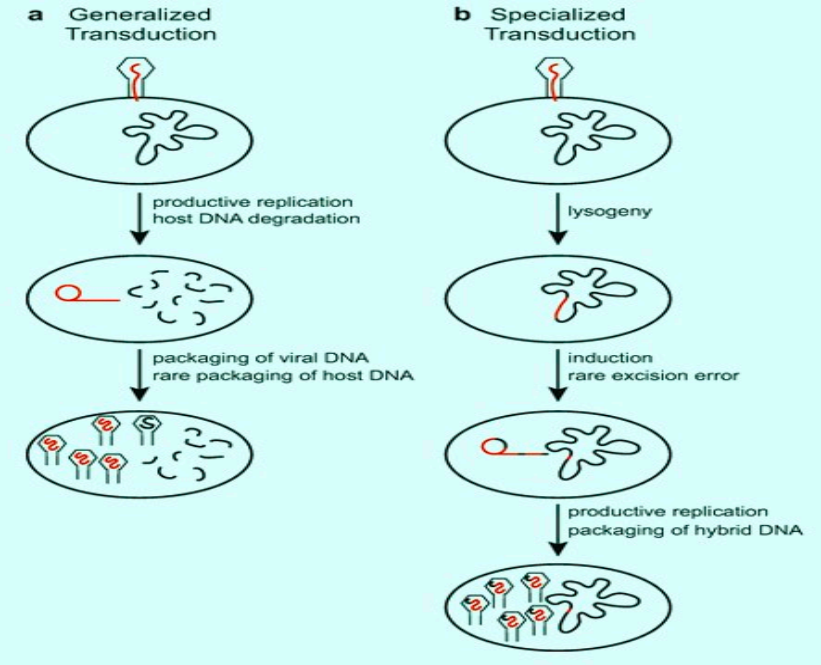

Figure 2. Generalized and specialized transduction responsible for ARGs transmission [32].

As per the latest study, through HGT, mobile genetic elements (MGEs) facilitate the transfer of resistance genes to non-resistant bacterial species, facilitating the accumulation and dissemination of antibiotic resistance genes in both gram-negative and gram-positive bacteria. It has been reported that phage particles can transduce genes (imipenem, aztreonam, and ceftazidime) in *Pseudomonas aeruginosa* [33], in *Staphylococcus epidermidis* (methicillin) [34], *S. aureus* (tetracycline), and can also disseminate genes from *Salmonella enterica* serovar *Typhimurium* DT10 [35]. The resistance genes for beta-lactamases encoded on bacterial chromosomes and plasmid can also be disseminated in gram-negative bacteria which are clinically important [36].

Phages Dissemination of ARGs via Transduction

The dissemination of antibiotic resistance genes (ARGs) can be achieved by both lytic and lysogenic phage cycles, with three distinct methods of phage-mediated transduction identified (Figure 3). Firstly, specialized transduction is facilitated by temperate phages, which unintentionally mobilize adjacent host genes during imprecise excision from the bacterial genome. Secondly, generalized transduction occurs when bacterial DNA, rather than phage DNA, is encapsulated within the phage head. This ability to package sizable DNA fragments enables transduction to indirectly facilitate the transfer of ARGs associated with other mobile genetic elements (MGEs). For instance, Zhang et al, demonstrated that T_4-like phages erroneously incorporated plasmid-borne ARGs through generalized transduction [37]. Transduction can also mediate the exchange of ARGs between different bacterial species. Studies have revealed that polyvalent phages can transfer ARGs between various *Enterococcus* and *Staphylococcus* species under controlled laboratory conditions [38].

Lastly, it has been a recently identified mechanism of phage-mediated transduction is lateral transduction. In this process, newly formed phage capsids efficiently package primarily bacterial DNA downstream of the phage insertion site. Lateral transduction stands out as the most potent mode of phage-mediated DNA transfer, capable of transporting several hundred kilobases and a broad section of the bacterial genome [39]. Unlike generalized transduction, which utilizes *ppac* sites, lateral transduction employs embedded *pac* sites for DNA packaging. Recently, Humphrey et al. (2021) conducted research utilizing *S. aureus* and *Salmonella* spp. as reference organisms [40], demonstrating that chromosomally encoded bacterial genes could be transferred at rates up to 1000-fold higher through lateral transduction compared to generalized transduction [41].

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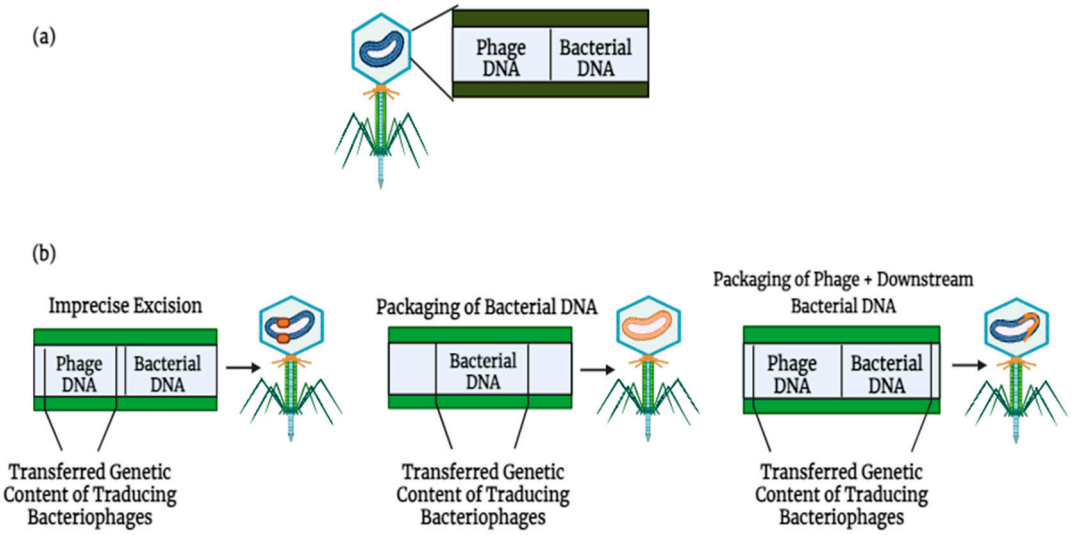

Figure 3. Lytic and lysogenic phages play a role in the development of bacterial antimicrobial resistance through various means: **(a)** Bacteriophages can harbor mobile genetic elements (MGEs) and facilitate the movement of antibiotic resistance genes (ARGs). **(b)** There exist three primary mechanisms by which phages facilitate the spread of genetic material [41].

Moreover, transduction frequencies of antibiotic resistance genes (ARGs) vary among different bacterial species and are influenced by various factors such as the efficiency of phage infection, the presence of suitable phage receptors on bacterial cell surfaces, and the mechanisms of gene transfer involved in transduction (Table 1). Some bacterial species may exhibit higher transduction frequencies for certain ARGs due to specific interactions between phages and host bacteria. Additionally, the genetic context of ARGs, such as their location within mobile genetic elements like plasmids or transposons, can affect their transduction rates. Understanding the transduction frequencies of different ARGs by various bacterial species is essential for elucidating the dynamics of antibiotic resistance dissemination in microbial communities and developing strategies to combat antimicrobial resistance.

Table 1. Variations in Transduction Frequencies of Different Antibiotic Resistance Genes (ARGs) Across Bacterial Species .

Bacteria	Phage	Antibiotic	Transduction frequency (transductants/pfu)	References
Clostridium difficile	ϕC2	Erythromycin	10^{-6}	[42]
Enterococcus	EGRM195	Tetracycline Gentamicin	10^{-8}-10^{-9} *(tet)* 10^{-7}-10^{-9} *(gent)*	[43]
Salmonella enterica serovar *typhimurium*	ES18	Tetracycline Chloramphenicol	10^{-8} *(tet)* 10^{-9} *(cam)*	[44]
Staphylococcal species	Φ80a and ΦJB	Penicillin Tetracycline	10^{-5}-10^{-6}	[45]
Staphylococcus aureus	80a	Streptomycin (in SaPi)	10^{-1}	[46]
Streptococcus pyogenes	nd	Tetracycline Chloramphenicol Macrolides	10^{-5}-10^{-6}	[47]

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		Lincomycin Clindamycin		

3. Bacterial Genes and ARGs in Viral Communities

Using outdated techniques in virology and bacteriophage studies has hindered the ability to culture phages within natural viral communities. Not being able to cultivate bacteriophages, various challenges have been encountered in assessing the characteristics of viral communities, such as their variety and the function of HGT in innate conditions. Metagenomic studies of viral communities in the human intestinal tract and wastewater plant-activated dirt deposits have confirmed the presence of genes for antibiotic resistance, including those encoding antibiotic efflux pumps, lipoproteins, TetC protein, streptogramin acetyltransferases, bleomycin, and β-lactamases within phage particles [48]. Analysis of sputum viromes from cystic fibrosis patients has revealed a significant abundance of genes related to antimicrobial resistance compared to non-cystic fibrosis cases. Specifically, 66 efflux pump genes, nine β-lactamase genes, and fifteen fluoroquinolone resistance genes have been identified. Phylogenetic studies have indicated that these resistance genes originate from different sources within the bacteriophage community of patients suffering from cystic fibrosis [49].

Recently in a few years, the main and powerful tools of genomic analysis have provided some knowledge to cover the different aspects of viral pollution. However, through metagenomics analysis, we gained much more information about the viral genomic materials that make up the viral populations found in the natural environment's biomass [50]. Metagenomics DNA can be sequenced through different sequencing techniques including Whole genome sequencing (WGS), which is better than the culture-dependent method. Viral metagenomics was first conducted in 2002, but before this, the PCR technique used to amplify the specific genes has made this possible to check the abundance of genes in the viral community, which is non-culturable. For instance, it was reported that bacteriophages infecting *E. coli* O157: H7 in wastewater reservoirs contained the gene for the Shiga toxin [51]. Similarly, viral communities present in activated plant sludge liquor contain bacteriophages that harbor sequences of 16S rRNA from various bacterial species. Furthermore, viral communities in raw municipal wastewater were found to contain sequences for blaOX A-2, blaPSE-1, or blaPSE-4, as well as blaPSE-type genes [52]. These findings collectively suggest that bacteriophages serve as reserves for ARGs in various environmental settings.

ARGs by Bacteriophages

Lysogenic bacteriophages, commonly found in clinical samples and natural environments, play a significant part in the transduction of ARGs, thereby facilitating the spread of antibiotic resistance. For instance, in *Streptococcus pyogenes*, tetracycline resistance genes, along with genes conferring resistance to antibiotics such as clindamycin, lincomycin, chloramphenicol, and macrolides have been transduced by bacteriophages. Additionally, erythromycin resistance genes have been induced in *S. pyogenes* transductants, making them more resistant to elevated erythromycin concentrations [53]. Additionally, in *S. pyogenes*, a chimeric genetic element consisting of a transposon inserted into a prophage is linked to *mefA* gene, which codes for a macrolide efflux protein [54]. A study carried out by Mazaheri and his coworkers reported that tetracycline-resistant genes were transduced from *E. gallinarum* to *Enterococcus faecalis* and gentamicin gene was transduced from *Enterococcus faecalis* to *Enterococcus faecium* and same gene was transduced from *Enterococcus faecium* to *Enterococcus casseliflavus* [55]. Table 2 displays the sources of antibiotics found in bacteriophage genomes.

Table 2. ARGs reported in the genome of bacteriophages or phages-like elements [56].

Antibiotic	Genes (Resistance)	Natural Source
Bacitracin	*bcr*A	Viromes of the human gut, microbiomes in fecal of swine, sputum microbiota of cystic fibrosis

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B-lactams antibiotics	*bla*$_{OXA-2}$, *bla*$_{PSE-1}$, *bla*$_{PSE-4}$, *bla*$_{PSE}$-type genes	Sewage
B-lactams antibiotics	*bla*$_{TEM}$, *bla*$_{CTX-M}$	Water from sewage, river, and animal waste
B-lactams antibiotics	*bla*$_{CTX-M-10}$	*Enterobacteriaceae*
Ampicillin	*bla*$_{CMY-2}$	*Salmonella enterica*
Trimethoprim	*dfrAa*	Microbiomes of Swine fecal
Fluoroquinolones	Fluoroquinolone resistance genes	sputum microbiota of cystic fibrosis
Macrolides	macB	microbiomes in fecal of swine
Methicillin	*mecA*	Water from sewage, river, and animal waste
Macrolides	*mef*A	*Streptococcus pyogenes*
Tetracycline	*tetW*	Viromes of human gut, microbiomes in the feces of swine
Tetracycline	*Tet37*	Microbiomes in fecal of swine
Tetracycline	*tetA*, *tetB*	*S. enterica*
Vancomycin	Vancomycin resistance genes	Viromes of the human gut, microbiomes in the feces of swine

4. Conclusion/Future Perspective

In conclusion, antibiotic-resistant microorganisms and ARGs are prevalent in our surroundings, indicating the likelihood of environmental sources contributing to resistance genes in human-associated microbial communities. Metagenomic sequencing analyses have revealed shared nucleotide sequences of MGEs between human pathogens and soil bacteria, underscoring the function of HGT mechanisms in their dissemination. Phages found naturally worldwide, serve as vehicles for ARG transfer, supplementing the traditional understanding of plasmid-mediated conjugation in HGT. Understanding and characterizing resistomes in natural and anthropogenic environments, including wastewater treatment plants contaminated with veterinary medicine, is crucial. Identifying specific biomes and bacteriophages involved in transferring ARGs among the infectious agents of humans and animals is essential for targeted interventions. Additionally, advancements in gene-editing tools like CRISPR-Cas offer opportunities for bioengineering phages to create broad-spectrum activities, potentially enhancing treatment efficacy. The convergence of antibiotics and bacteriophages represents an unexplored frontier in ARG research, warranting further investigation. Additional research endeavors are required to thoroughly examine the spread and emergence of ARGs, given their implications for public health. It is essential to investigate the impact of these contacts on the expansion of antibiotic tolerance and resistance.

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Abbreviations

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ARG	Antibiotic resistance genes
HGT	Horizontal gene transfer
MGEs	Mobile genetic elements
WHO	World Health Organization
CDC	Center For Disease Control
VBR	Virus-to-bacterium
VLPs	Virus-like particles

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